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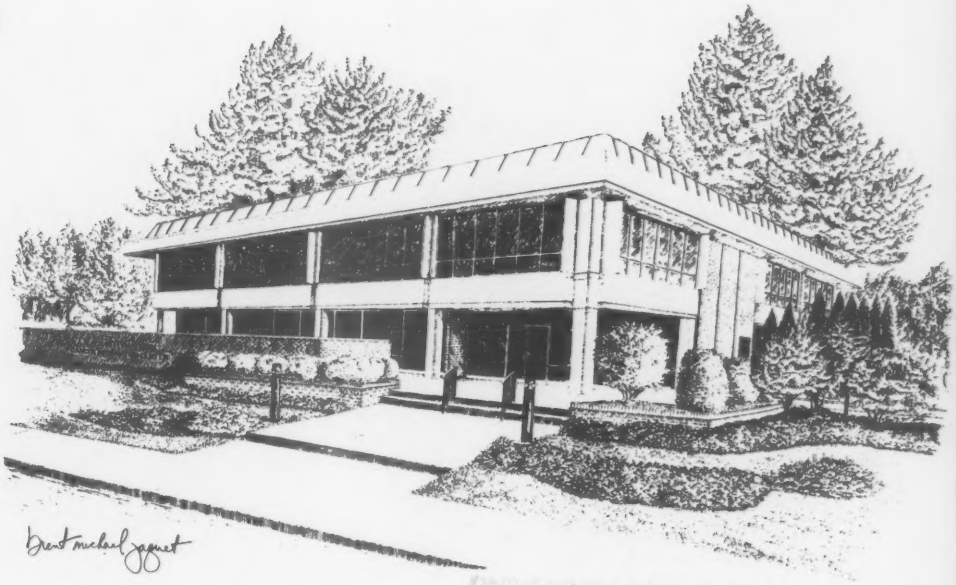

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1059 ANNOUNCEMENTS

Treatment of Chemotherapy Extravasation: Current Status[1,2]

Kerry VanSloten Harwood* and Joseph Aisner[3]

As the benefit of chemotherapeutic treatment of cancer is further defined and the role of this modality is expanded, attention must be given to prevention and treatment of its associated side effects. Debilitating toxicity as a result of chemotherapy may delay treatment or even lead to patient refusal of continued therapy. Many chemotherapy agents have the potential for local toxicity. This toxicity may be expressed in one of several ways. Some agents may be irritants, producing pain upon administration or phlebitis which may be severe enough to prevent further usage of the vein. Some agents produce a hypersensitivity reaction. In this category, at least three agents (doxorubicin, daunorubicin, and mechlorethamine) have been seen to produce local hypersensitivity manifested by urticaria or erythematous streaking with or without pruritis or pain (1-4). This is generally a local reaction with only one case of anaphylaxis reported (1). A number of chemotherapy agents may be vesicants, producing toxicity when tissue extravasation occurs. The sequelae of tissue extravasation of this group of agents range from mild induration and erythema to severe necrosis of dermis and underlying structures requiring skin grafting or even amputation of the affected limb.

The incidence of local toxicity is difficult to discern from the literature. The incidence and severity of phlebitis are rarely reported. The incidence of hypersensitivity reactions with doxorubicin has been reported in one sample as 3% (4) and in another as 21% (1). Identical reactions with daunorubicin can be seen. Hypersensitivity with mechlorethamine and aclarubicin has been documented only by case report (4,5). The incidence of extravasation necrosis can only be inferred from what is reported in the literature regarding the most frequently used vesicant, doxorubicin. The incidence of extravasation necrosis has been reported as up to 6% of patients treated (6,7) in large patient samples. Due to variability in identification, severity, and treatment, the natural course of extravasation is also difficult to discern. However, in Larson's (8) sample of 50 patients with extravasation of doxorubicin, vincristine, or both treated initially with ice and elevation, 12 required surgical intervention for pain or skin necrosis. In agents such as doxorubicin or vincristine, the problem has been reasonably well-defined. Other agents, also widely used, such as cisplatin and etoposide, have only recently been identified to have potential to produce extravasation necrosis (9,10). In addition, experimental agents may come into phase I or II testing with the potential for severe problems and with little if any information regarding local toxicity.

Because the information defining local toxicity of various chemotherapy agents is scant, information regarding effective treatment is similarly lacking or anecdotal at best. The problems of differing mechanisms of tissue damage, inadequacies in the animal models, and inability to effectively utilize the clinical situation for research have thwarted the development of effective treatments for local chemotherapy toxicity. This paper will define chemotherapy agents with local toxicity and will review the research methods and treatment modalities as currently known.

Local Irritation

As seen in table 1, many of the agents which produce either phlebitis or hypersensitivity reactions are also vesicants if extravasated. However, any local reaction, regardless of drug involved, should be carefully categorized to determine appropriate intervention or to develop sufficient data regarding type and severity of reaction, and response to any interventions. The pain and phlebitis associated with an irritant may be due to a local effect on the vessel producing spasm and pain. Initially, this may not be discernable from extravasation. The spasm, in turn, may be an obstacle to flow, increasing the risk of extravasation. Other agents can produce irritation of the vessel endothelium with a chemical burn reaction (fig 1). Another irritant effect may be related to the insolubility of the drug, as with bisantrene (11), or by the effect of the diluent, as seen with carmustine. There is no recognized treatment. These irritant effects may be alleviated

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[3]University of Maryland Cancer Center, University of Maryland Hospital, Baltimore.
* *Reprint requests to:* Kerry VanSloten Harwood, RN, Johns Hopkins Oncology Center, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 1.—Chemotherapy agents with known capacity for local toxicity

Vesicants	
Cisplatin	Mitomycin
Dacarbazine	Streptozocin
Dactinomycin	Teniposide
Daunorubicin	Vinblastine
Doxorubicin	Vincristine
Etoposide	Vindesine
Mechlorethamine	Zinostatin
Mithramycin	Zorubicin
Mitoguazone	

Irritants	
Phlebitis	*Hypersensitivity*
Bisantrene	Aclarubicin
Carmustine	Daunorubicin
Mechlorethamine	Doxorubicin
	Mechlorethamine

by the initial use of large bore veins with rapid fluid infusion allowing for dilution of the irritant. In the event of phlebitis or sclerosis of vein, other sites should be sought. The irritant effect may cause venous access problems but should not otherwise contraindicate use of the drug. Because at least one of the recognized phlebitis-producing irritants, mechlorethamine, is known to produce extravasation necrosis, careful assessment and proper categorization must take place at the first indication of pain.

Hypersensitivity Reaction

A similar careful differentiation must be made when evaluating a hypersensitivity reaction, because the four agents known to cause hypersensitivity reactions also have the potential for extravasation necrosis. Hypersensitivity reactions, sometimes described as "flare" reactions, are characterized by an erythematous streak, urticaria, or both along the course of the vein (4). Pain or pruritis may be present. Pain associated with hypersensitivity reactions has been described as a dull ache along the course of the vein. In contrast, localized burning or stinging either at the injection site or along the vein proximal to injection site is more characteristic of leakage into tissue. Extravasation may occur proximal to the injection site where the vein wall is weakened, possibly by previous venipuncture, and may be recognized as such by the more localized symptoms. The symptoms of hypersensitivity reactions usually resolve spontaneously within 30

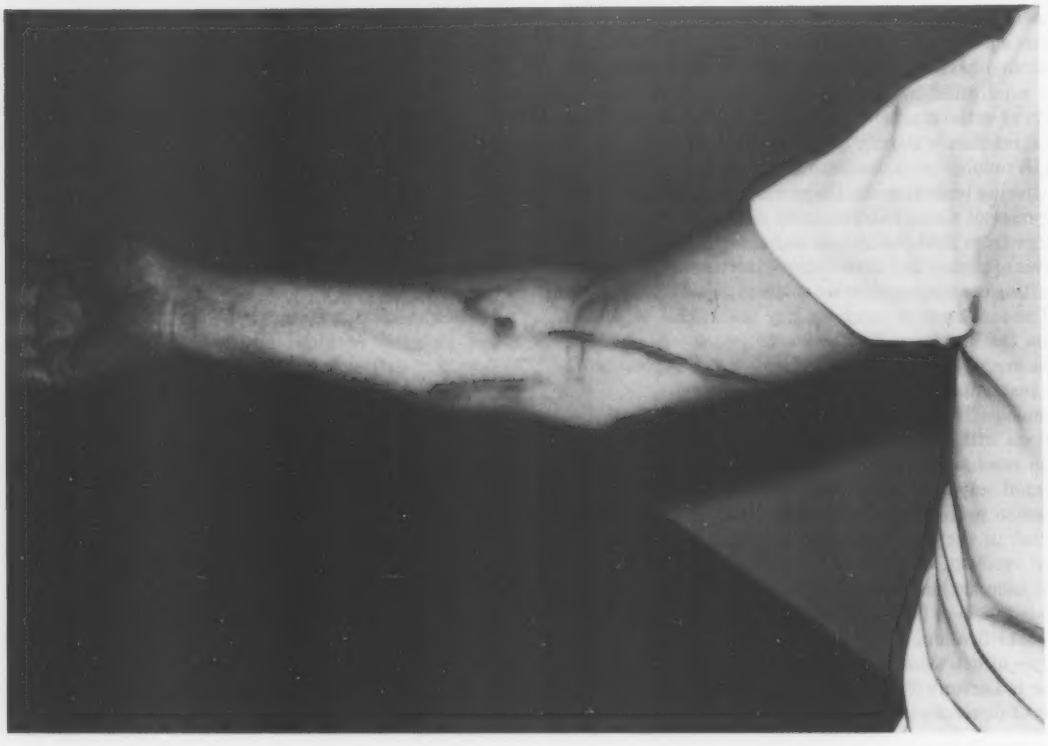

FIGURE 1.—Example of local irritant effect: patient with chemical burn from mechlorethamine as part of MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) chemotherapy, photographed 2 mos after completing 6th course.

minutes. Anaphylaxis has only been reported once (1). Antihistamines have been reported to be unsuccessful in consistently preventing hypersensitivity (1). Reinitiation of drug administration in a new site may not result in repeat of the hypersensitivity reaction.

Tissue Extravasation

The suspicion of tissue extravasation, as opposed to venous irritation or local hypersensitivity, is not always based on the patient's symptoms. The patient may immediately experience pain, erythema, or swelling at the site of extravasation, or the only indication may be a decrease in flow rate or lack of blood return. Early detection of extravasation of vesicant agents is critical in preventing further administration of drug and in quickly implementing appropriate measures, when known. The chemotherapeutic agents known to be vesicants can be divided into three categories: those with purported effective treatment measures seen in clinical case reports or animal studies, those with purported antidotes described in the literature without the benefit of clinical or animal testing, and those with no effective treatment reported. Those agents with clinical or laboratory basis for treatment of extravasation are listed in table 2.

The majority of potential antidotes for chemotherapy extravasation have been tested for doxorubicin. At least five different interventions have been reported to have some degree of efficacy in alleviating extravasation toxicity in either animal models or clinical cases. However,

TABLE 2.—Vesicant chemotherapy agents with recommended antidotes based on clinical or laboratory experience

Drug	Purported antidotes	Positive effects in—		Negative effects reported
		Animal models	Clinical case reports	
Doxorubicin	Hydrocortisone 50–200 mg sc or intradermally Rationale: decrease inflammation	No (refs 15, 21, 24)	Yes (ref 6)	No
	Sodium bicarbonate 8.4%; 5 ml through iv line Rationale: decreased DNA binding by pH change	Yes (ref 15)	Yes (ref 18)	Yes (ref 27) Skin necrosis if too much given
	DMSO topically Rationale: free-radical scavenging	Yes (refs 19, 20) No (ref 22)	Yes (refs 29, 30)	No
	DMSO + alpha tocopherol; 10% alpha tocopherol in 1 ml DMSO topically daily for 2–7 days Rationale: free-radical scavenging	Yes (ref 21) No (refs 20, 22)	No	Yes (refs 17, 22) Increased toxicity if DMSO given intradermally
	Isoproterenol HCl 10 mg intradermally or propanolol HCl 100 mg intradermally Rationale: prevent toxic reaction of doxorubicin with skin beta-receptors	Yes (ref 23)	No	No
	Cold	Yes (ref 14)	Yes (ref 8)	No
Daunorubicin	*See* doxorubicin			
Mechlorethamine	10% sodium thiosulfate; 4 ml through iv line Rationale: inactivation by alkalination	Yes (ref 28)	No	No
Vinblastine	Hyaluronidase 1 ml sc Rationale: drug dilution	Yes (ref 25)	No	No
	Normal saline 1 ml sc Rationale: drug dilution	Yes (ref 25)	No	No
	Hydrocortisone 2.5 mg sc Rationale: decrease inflammation	No	No	Yes (ref 25)
Vincristine	Normal saline 1 ml sc Rationale: drug dilution	Yes (ref 16)	No	No
	Hydrocortisone 25 mg sc Rationale: decrease inflammation	Yes (ref 16)	No	No
Vindesine	*See* vinblastine			

there frequently remains a sense of urgency when extravasation occurs. It is frequently feared that even minimal extravasation can lead to a severe necrotic ulcer (fig 2). Necrosis does not always result from small extravasations and there is no clear idea regarding the natural course of most extravasations. There are minimal data in man linking dose and degree of skin toxicity. Several animal models have been used, including the mouse, rat, and pig, and results are not consistent between models. Although pigskin is similar to human skin, the animal's size and expense have discouraged large-scale testing. The mouse or rat is a cheaper, more convenient model and has been used extensively. However, an inherent difference between rodent and human skin, the presence of the panniculus carnosus, makes extrapolation from this model difficult. This thin muscle layer, adherent to the skin, also provides the blood supply to the skin. When doxorubicin is injected intradermally, above the panniculus carnosus, necrosis is consistently produced. The necrosis, however, is not as progressive as that sometimes seen clinically and heals without treatment in 6–8 weeks (10,12).

The treatment of doxorubicin extravasation with steroids to reduce the initial inflammation has been advocated since 1978, primarily based on the work of Barlock et al (6). In this clinical study, comparison of hydrocortisone given through the iv needle, versus sc and intradermally, showed negligible difference with none of nine extravasations developing necrosis. However, steroids have not shown any benefit in decreasing doxorubicin extravasation necrosis when carefully tested in a mouse model (13). Barlock's clinical investigation used local cooling in all patients. This uncontrolled factor may have been the active intervention. Dorr and Alberts (14) recently demonstrated significant amelioration of doxorubicin necrosis in a mouse model using only local cooling. Most reported clinical cases have used local cooling as an uncontrolled intervention. In Larson's (8) report of extravasations, 76% of patients were treated successfully with ice and elevation, but no differentiation was made between doxorubicin and vincristine extravasations. This may account for the 24% of patients who did develop extravasation necrosis. The use of sodium bicarbonate as an antidote to doxorubicin extravasation has been explored, based on the pH dependency of doxorubicin tissue binding. The sodium bicarbonate is re-

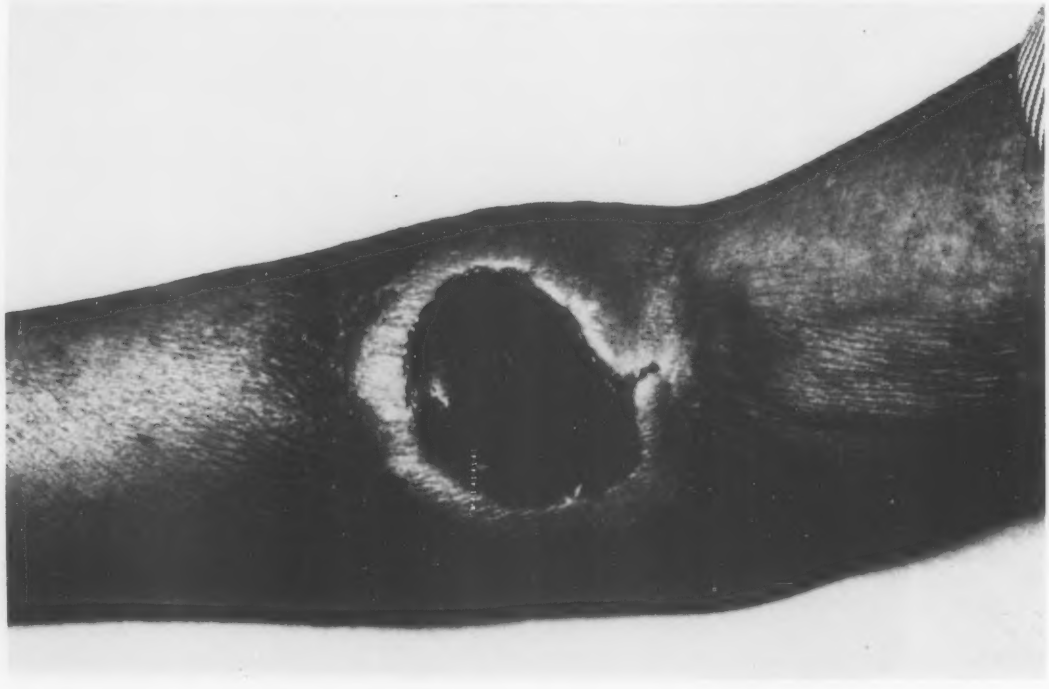

FIGURE 2.—Example of extravasation necrosis: patient with necrosis and scar formation secondary to extravasation of daunorubicin, photographed 4 wks after extravasation.

ported to decrease, although not prevent, toxicity in a rat model (15). However, this has not been substantiated in other animal studies (16,17). Clinically, $NaHCO_3$ has been reported to be effective when used in combination with steroids, but this has not been substantiated using appropriate controls (18).

Another approach has been to use compounds which appear to interact with doxorubicin. DMSO, alpha tocopherol, and the combination have been tested as potential antidotes with inconsistent results. Desai and Teres (19) reported complete prevention of necrosis in the rat model with either intradermal or topical DMSO, with an absence of DMSO local toxicity. They reported complete prevention of necrosis at the treatment site, and also at a distant untreated site in a pig model using 7 days of topical DMSO (20). DMSO alone was ineffective in preventing necrosis in the rat model described by Svingen et al (21), but in combination with alpha tocopherol reduced the ulcer diameter by 68%. In this experiment, untreated injection sites on the treated animal were, again, significantly smaller than sites on the control animal ($P < 0.05$). In contrast with these studies, intradermal DMSO has been reported to produce erythema in a mouse model and, given after doxorubicin, apparently increased toxicity (22). Alpha tocopherol alone has been tested in the mouse and rat models with negative results in both (20,22). The combination has also been reported to be ineffective in a mouse model (22).

The diverse results obtained with these interventions suggest that further study is needed to determine the role of each. Considering the current data, the use of the pig model would seem advisable for most applicability to the clinical situation. Furthermore, the use of control animals in addition to control sites would seem important due to the apparent systemic effect. For DMSO, all reports have utilized a standard 7-day treatment period and standard doxorubicin injection, but the dose of DMSO has varied, ranging from 0.5 ml of 70% DMSO to 3 ml of 100% DMSO, and this may account for the noted divergence. Thus, in addition to controlling for animal model and vesicant doses, the antidotes also needed to be carefully controlled. Dorr and Alberts (23) have also reported amelioration of doxorubicin toxicity in the mouse model using the beta-adrenergic compound isoproterenol (partial relief) and the beta-adrenergic antagonist propanolol (complete prevention with low-dose extravasation). This effect, however, has not been investigated in a pig model.

The list of potential antidotes described as ineffective in treating doxorubicin extravasation in an animal model now includes lidocaine, bupivacaine, phentolamine, hyaluronidase, cimetidine, diphenhydramine, sodium heparin, *N*-acetylcysteine, benzoic acid, glutathione, coenzyme Q10, and carnitine (13,16,17,23,24).

Investigation of treatment for extravasation of vinca alkaloids, although not as extensive as that of doxorubicin, has provided some management clues. In the mouse model (25), hyaluronidase and normal saline were both antidotal following extravasation of vinca alkaloids. Either promoting drug absorption (hyaluronidase) or providing drug dilution (normal saline) being effective would suggest that heat (suggested by manufacturer) to promote absorption may be effective. This has yet to be demonstrated either clinically or in an animal model.

In addition to the interventions with some laboratory or anecdotal basis for clinical use, there are many rationally designed potential treatments that have not had sufficient testing to determine efficacy (table 3). There are no known or theoretical antidotes for most of the vesicants. Certainly, this is an area for further investigation.

Considering the paucity of established treatments, the best approach is prevention. The following general guidelines can be used to minimize the number of chemotherapy extravasations:

(*a*) Administration of chemotherapy should be performed only by individuals familiar with its toxic effects.

(*b*) The site of administration is selected with consideration of visualization, vessel size, and potential damage if extravasation occurs, the order of preference being forearm, dorsum of hand, wrist, and finally, antecubital veins. The arm on the side of a radical mastectomy is generally avoided. A central venous line can be utilized if necessary for safe administration.

(*c*) "Butterfly" or plastic cannula needles are used peripherally and inserted by the person administering chemotherapy to ensure against multiple venipuncture.

(*d*) The needle is taped in place, but the needle entry site is either left uncovered or a see-through sterile dressing is applied to allow visualization.

(*e*) A running iv of either 5% dextrose in water or normal saline is maintained. After observation of flow rate, blood return, and site, administration is initiated into the tubing with the iv running by gravity to assess for back pressure.

TABLE 3.—Vesicant chemotherapy agents lacking tested antidotes

Drug	Proposed antidote
Cisplatin	None
Dacarbazine	None
Dactinomycin	None
Etoposide	None
Mithramycin	EDTA (ref 28)
Mitoguazone	None
Mitomycin	Sodium thiosulfate (ref 28)
Streptozocin	None
Teniposide	None
Zinostatin	None
Zorubicin	None

(*f*) The patient is questioned about discomfort; blood return, flow rate, and iv site are checked frequently during administration.

(*g*) The line is flushed with at least 10 ml of the iv fluid before discontinuation to prevent backspill of drug.

(*h*) If discomfort, swelling, or redness is noted, the medication is immediately stopped.

Despite careful adherence to these procedures, chemotherapy extravasation occasionally occurs. The current literature does not establish any particular treatment as "standard" for any specific chemotherapy extravasation, and further animal and clinical studies are clearly needed.

If it appears that an extravasation has occurred, then it is necessary to have a regular routine to allow for prompt intervention. One such routine might be:

(*a*) The iv needle is left in place while deciding if a potential antidote is to be injected. Flow of fluid into needle is discontinued. Any drug remaining in the tubing is removed.

(*b*) If there is an obvious bleb of extravasated drug, it is aspirated with a 25-gauge needle.

(*c*) For extravasations involving doxorubicin or daunorubicin and most other agents, an ice pack is applied immediately. For vinca alkaloids, heat is applied.

(*d*) A potential antidote, if appropriate, is then injected through the iv needle. Following the administration of the antidote, the needle is removed. Sc, intradermal, or topical antidotes are given if appropriate.

(*e*) Recording of events should include approximation of amount of extravasated drug, time to intervention, type of intervention, and measurement of any lesions, with photographs if possible.

If both prevention and early intervention are not successful and extravasation necrosis does occur, then there is the need for further careful care and observation. The proper management under these circumstances is not well-defined but early surgical intervention may well be appropriate (12,26). This is particularly true for the anthracyclines, where there may be release of parent compound and active metabolites from injured tissue. Thus, debridement and wide excision, including erythematous rather than only necrotic tissue, may limit the size of the injury. In addition, careful observation for local infection or possibly early institution of appropriate (broad spectrum) antibiotics will improve the chances of limiting the size of the damage. When such measures fail, extensive surgery, including amputation, may become necessary.

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Doxorubicin, Bleomycin, Vinblastine, and Dacarbazine (ABVD) Salvage of Mechlorethamine, Vincristine, Prednisone, and Procarbazine (MOPP)-Resistant Advanced Hodgkin's Disease[1]

Andrea Piga,[2,*] Achille Ambrosetti,[3] Giuseppe Todeschini,[3] Gianluigi Cetto,[3] Giuseppe Perona,[3] and Riccardo Cellerino[2,4]

Thirty-nine patients with advanced Hodgkin's disease were treated with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD). Thirty-one of these patients had previously failed to respond to MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) treatment (29 patients) or to CCVPP (lomustine, cyclophosphamide, vincristine, procarbazine, and prednisone) treatment (two patients). Twenty-seven patients were considered evaluable: eight (30%) achieved complete remission (CR), two (7%) achieved partial remission (PR), and 17 (63%) had no response. All of the complete responders are still in continuous unmaintained CR, while 14 of the 19 partial responders and nonresponders have died. The overall median survival was 21 months; it was 16 months for partial responders and nonresponders. The remaining eight patients had ABVD substituted to MOPP early in treatment, because of allergy to procarbazine: six patients achieved CR and two achieved PR. Our results are in keeping with the literature data and confirm the effectiveness of ABVD as primary and salvage treatment in advanced Hodgkin's disease. [Cancer Treat Rep 68:947–951, 1984]

The management of patients with advanced Hodgkin's disease (HD) who fail to respond to primary treatment or relapse after achieving a complete response (CR) is still unsatisfactory. Unfortunately, this concerns about one-half of the patients with advanced disease, since only 54% of treated patients have long-term, disease-free survival (1). With the aim of offering a second chance of effective treatment to patients who have failed or relapsed after MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) therapy, various new combinations have been designed employing drugs with proven effectiveness against HD when used as single agents.

The percentage of CRs in previously untreated patients treated with ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) was comparable to that in similar patients treated with MOPP; a lack of cross-resistance between the two regimens was also reported (2). Therefore, ABVD could represent successful treatment for patients with MOPP resistance.

We have evaluated the effectiveness of ABVD in 27 MOPP-resistant patients with advanced HD and in an additional eight patients in whom MOPP treatment was discontinued because of allergy to procarbazine.

PATIENTS AND METHODS

Thirty-one patients with advanced, MOPP-resistant disease were entered in the study between August 1975 and April 1982. Twenty-seven patients were evaluable as of March 1983. The four unevaluable patients were excluded for the following reasons: death (not drug-related) during the first course of therapy (one patient), sporadic administration of dacarbazine (one), and refusal to continue treatment after two courses (two). Criteria for patient entry in the study were progression of disease while receiving MOPP or PR or no response after MOPP induction. Two patients receiving CCVPP (lomustine, cyclophosphamide, vincristine, procarbazine, and prednisone) (3) as primary treatment were also included. Only one patient had previously received doxorubicin as part of MABOP (mechlorethamine,

[1]Received June 13, 1983; revised Jan 12, 1984; accepted Feb 1, 1984.
[2]Cattedra Oncologia, University of Ancona, Italy.
[3]Cattedra Ematologia, University of Verona, Italy.
[4]We are indebted to Mr. Massimiliano Grilli for secretarial help.
[*]*Reprint requests to:* Dr. Andrea Piga, Cattedra Oncologia Clinica, Osp. Torrette, 60100 Ancona, Italy.

TABLE 1.—Clinical characteristics of evaluable patients and response to ABVD

Patient No.	Age (yrs)	Sex	Histology*	Stage, A/B symptoms	Sites of extranodal involvement	Prior treatment (No. of MOPP or CCVPP courses)	Response to MOPP (or CCVPP)†	Time from diagnosis to ABVD (mos)	No. of ABVD courses	Response†	Survival from start of ABVD (mos)‡
1	22	F	LP	IVA	Lung	MOPP (6)	PR	10	6	CR	70+
2	48	F	NS	IIIB	None	MOPP (3)	NR	4	6	NR	10
3	70	M	MC	IVB	Lung	MOPP (4)	NR	5	3.5	NR	6
4	26	M	MC	IVA	Bone	MOPP (6)	NR	10	6	NR	17
5	65	M	LP	IIIB	None	MOPP (5)	PR	8	6	NR	9
6	38	F	MC	IVB	Bone	MOPP (6)	PR	9	6	CR	65+
7	38	M	MC	IIIB	None	MOPP (6)	PR	9	5	NR	20
8	47	F	NS	IIIA	None	MOPP (6)	PR	11	3.5	NR	12
9	66	F	MC	IVB	Bone	MOPP (6)	NR	8	5	PR	16
10	72	F	MC	IIIB	None	IF + MOPP (5)	NR	22	5.5	CR	18+
11	64	M	NS	IVB	Lung	MOPP (5)	NR	13	3	NR	5
12	22	M	MC	IVB	Bone	MABOP + IF + MOPP (6)	NR	57	6	NR	12
13	38	M	MC	IVB	Liver	MO(P)P§ (6)	NR	13	6	PR	38
14	34	M	MC	IVB	Bone/CNS	IF + MOPP (8)	NR	156	3	NR	7
15	37	M	NS	IVB	Lung	MOPP (6)	NR	9	6	CR	60+
16	24	M	MC	IVB	Bone/CNS	MOPP (6)	NR	7	2.5	NR	6
17	28	F	MC	IIIB	None	MOPP (6)	NR	11	4	CR	44+
18	26	M	MC	IIIB	None	MOPP (6)	NR	9	6	NR	15+
19	16	F	NS	IVB	Bone/liver	MOPP (6)	NR	7	6	NR	23+
20	59	M	NS	IVB	Bone	MOPP (6)	NR	10	4.5	NR	11+
21	69	F	LD	IVA	Gastrointestinal tract	MOPP (4)	NR	6	2	NR	2
22	40	M	NS	IIIB	None	MOPP (8)	PR	13	6	CR	12+
23	35	M	LP	IVB	Bone	MOPP (6)	PR	11	5	CR	11+
24	70	F	NS	IIIB	None	MOPP (5)	NR	10	4.5	NR	11+
25	37	F	MC	IIIA	None	MOPP (2)	NR	6	6	NR	47+
26	23	M	NS	IIB	None	CCVPP (6)	PR	11	2.5	CR	24+
27	41	M	NS	IVB	Bone	CCVPP (8)	NR	15	5.5	NR	21

*LP = lymphocytic predominance; NS = nodular sclerosis; MC = mixed cellularity; and LD = lymphocytic depletion.
†PR = partial response; and NR = no response.
‡+ = patient is still alive.
§MO(P)P = procarbazine not given because of intolerance.

doxorubicin, bleomycin, vincristine, and prednisone) (4). Three patients had also received involved-field radiation therapy (IF). Clinical characteristics of evaluable patients are shown in table 1. An additional eight patients had ABVD substituted for MOPP because of their intolerance to procarbazine.

Entry in the study was preceded by accurate evaluation of the disease stage; 1 month after the ABVD treatment, an accurate restaging was carried out. Staging and restaging procedures included routine clinical and laboratory examinations, bone and liver/spleen scans, bone marrow aspirate, and trephine and liver biopsies; lymphangiography and other examinations necessary to evaluate areas of suspect involvement were performed only before entry in the study and were not repeated at the end of treatment unless initially positive.

Six courses of treatment were given unless progression of disease or severe toxicity was evident. ABVD was administered as previously described (2) or, mostly, with dacarbazine at a dose of 375 mg/m^2 on Days 1 and 15. The suggested dosage reduction schedule (2) was adopted; however, in patients with leukopenia/thrombocytopenia, a 1-week delay was usually preferred to dosage reduction. CR was defined as the complete absence of signs or symptoms attributable to HD; PR was defined as a significant improvement of clinical conditions with no systemic B symptoms and at least a 50% reduction in size of measurable disease; and no response was defined as a reduction of < 50% or disease progression during treatment.

Therapy was discontinued after achievement of CR or PR; different treatments were employed at relapse or when progression occurred during the administration of ABVD.

RESULTS

MOPP-Resistant Patients

Of 27 evaluable patients eight (30%) achieved CR, two (7%) achieved PR, and 17 (63%) had no response. Patients achieving CR are still in unmaintained continuous CR after 4, 5, 11, 22, 38, 54, 59, and 62 months from completion of ABVD. Fourteen of 19 patients defined as having PR or no response have died. The overall median survival was 21 months, and the median survival of patients with PR or no response was 16 months (fig 1). No correlation was evident between CR and sex, histology (although two of three patients with lymphocytic predominance achieved CR), stage, A/B symptoms, or time from diagnosis to ABVD treatment. Seven of 16 patients ≤ 40 years of age and one of 11 > 40 years of age achieved CR. Five

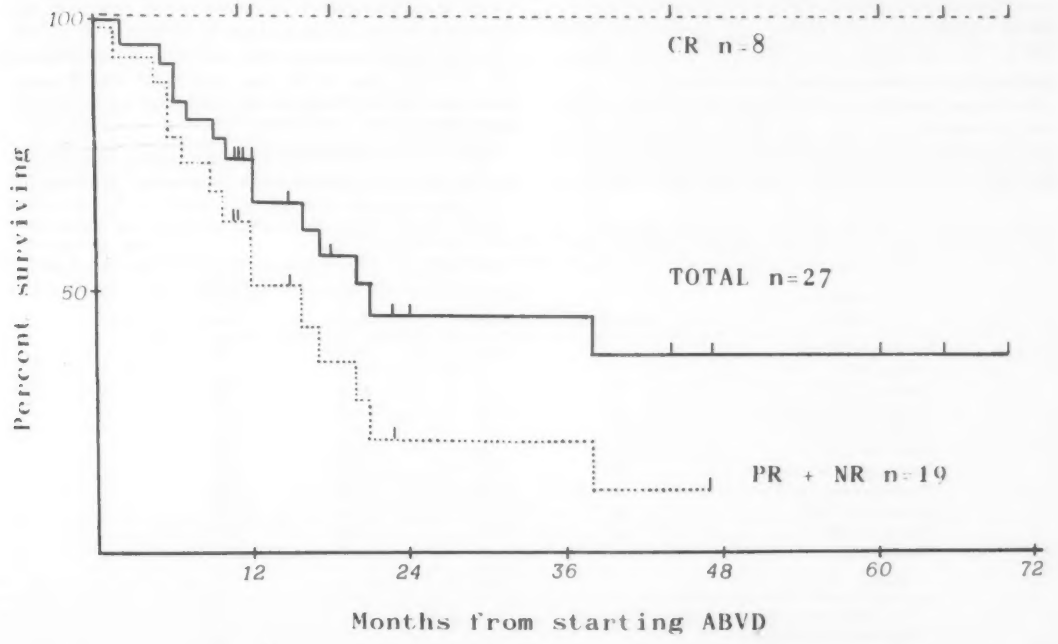

FIGURE 1.—Actuarial survival from the initiation of ABVD treatment according to response.

of eight patients achieving PR after primary treatment and three of 19 with no response achieved CR after ABVD.

Patients With Intolerance to Procarbazine

Six of eight patients in this group achieved CR and are still alive (and in continuous unmaintained CR) after 10, 23, 23, 26, 31, and 40 months. Two patients achieved PR and are still alive and without evidence of disease progression at 8 and 9 months.

Toxicity

The chemotherapy regimen was well-tolerated. Side effects like nausea and vomiting, moderate alopecia, and transient febrile episodes were common; chemical phlebitis and mild skin hyperpigmentation in the sites of skin stress were not unusual.

Myelosuppression was moderate. Only one patient had prolonged thrombocytopenia (platelet count 25 × 10^9/L); in 12 patients, leukopenia/thrombocytopenia caused a sensible delay in the interval between courses. The mean duration of a course was 37.3 days. The patients with MOPP-resistant disease received mean doses of the drugs comparable to those of procarbazine-intolerant patients and to those reported by Bonadonna et al (2) for untreated patients (indicated between parentheses): doxorubicin, 79% (87%); bleomycin, 94% (96%); vinblastine, 81% (87%); and dacarbazine, 87% (96%). No correlation was observed between hematological toxicity and response to treatment.

Cardiac or pulmonary disturbance or relevant peripheral neuropathy was not observed. One patient developed a massive nonlethal generalized varicella zoster at the end of chemotherapy, one developed localized herpes zoster, and one developed local urticaria following dacarbazine infusion.

DISCUSSION

Many regimens have been employed in the treatment of MOPP-resistant, advanced HD. Most of these regimens include drugs which are different from the four employed in the MOPP regimen. The best results (> 50% CR) have been reported with B-DOPA [bleomycin, dacarbazine, vincristine, prednisone, and doxorubicin (5)], B-CAVe [bleomycin, lomustine, doxorubicin, and vinblastine (6)], and with the combination of doxorubicin and lomustine (7). However, the number of patients studied is small and definitive conclusions are not possible. After the first report the ABVD regimen has been used by various investigators, with contrasting results (table 2). The heterogeneity of these studies with regard to various clinical characteristics (duration of disease, histology, stage, previous treatment, etc) may partly account for this discordance. Initial response to MOPP and time to relapse after MOPP-induced CR seem to be the more relevant factors in determining the clinical outcome of subsequent treatment. By re-treatment with MOPP, Fisher et al (17) obtained a 93% CR rate in patients who relapsed after at least 12 months after MOPP-induced CR, while this percentage decreased considerably for patients with duration of remission < 12 months (only five of 17 patients achieving CR). In a study by Krikorian et al (10), six of 27 patients treated with ABVD achieved CR, but this included none of 12 who had failed MOPP treatment or relapsed within 3 months from achieving CR with MOPP.

Eight of 27 MOPP-resistant patients achieved CR and six of eight patients with intolerance to procarba-

TABLE 2.—Response to ABVD according to various studies

Investigator(s), year	Ref. No.	No. of patients	CR (%)	Median survival* (mos)
Vicente and Cortes-Funes, 1976	8	15	5 (33)	NA
Case et al, 1977†	9	24	1 (4)	NA
Krikorian et al, 1978†	10	27	6 (22)	22+
Sutcliffe et al, 1979‡	11	41	3 (7)	12
Amiel et al, 1980†	12	22	7 (32)	NA
Bauters et al, 1980	13	21	9 (43)	30+
Gassmann et al, 1981§	14	19	7 (37)	NA
Santoro et al, 1982	15	54	32 (59)	27
Papa et al, 1982	16	18	10 (55)	38
Present study	—	27	8 (30)	21
Totals		268	88 (33)	

*+ = median not reached at the time of the study. NA = not available.
†Modified ABVD regimen.
‡MOPP-resistant patients. Vincristine was often substituted for vinblastine.
§C-MOPP-resistant patients.

zine but not MOPP-resistant achieved CR.

Other prognostic variables are more difficult to detect when dealing with small numbers of patients. For example, our patients ⩽ 40 years of age did much better (seven CRs among 16 patients) than older patients (one CR among 11 patients); the reverse was true in the Milan series (15).

Our MOPP-resistant patients are a relatively homogeneous group, with recent onset of the disease, absence of extensive previous chemotherapy or radiotherapy, and previous treatment not employing drugs included in the ABVD regimen. The overall results confirm the effectiveness of ABVD in the salvage of MOPP-resistant patients. We have obtained a percentage of long-lasting unmaintained CRs and an overall median survival quite unexpected in a group of patients with a particularly poor prognosis (no response or at best a PR to primary treatment), and this without significant toxicity.

In the absence of a large controlled clinical study which compares ABVD with other regimens (eg, B-CAVe, etc), we consider ABVD, the effectiveness of which is definitely confirmed by a large series of literature data, the first choice of treatment in the salvage of MOPP-resistant, advanced HD. In addition, procarbazine-intolerant patients, treated by us in the past with the omission of procarbazine from MOPP, have unaltered chances of achieving CR if crossed to ABVD treatment.

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Improved Survival Among Children With Acute Leukemia Diagnosed in the 1970s[1]

Sandra C. Steinhorn[2,3,*]

Improvements in survival were observed among white children < 15 years of age diagnosed with acute lymphocytic leukemia (ALL) between 1973 and 1978 in nine of the areas covered by the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program. Significant increases were noted between the 3-year survival rate for children diagnosed in 1973–1975 (59%) and the rate for those diagnosed in 1976–1978 (74%). The 5-year survival rate for children diagnosed with ALL in 1973–1975 was nearly 50%. Survival of female children exceeded that for males, and younger children 1–4 years of age diagnosed with ALL had a better prognosis than older children 5–14 years of age. Both sexes and age groups showed increases in survival between 1973–1975 and 1976–1978. Improvements in survival during these time periods were not observed for children diagnosed with acute granulocytic (myelogenous) leukemia. Between 1973 and 1979, the age-adjusted incidence rate for all childhood leukemias remained fairly constant in the SEER areas, ranging from 3.7 per 100,000 population < 15 years of age in 1973 to 3.5 per 100,000 in 1979. Reflecting observed improvements in childhood leukemia survival, the corresponding mortality rates fell from 2.2 to 1.7 per 100,000 in this same time period. [Cancer Treat Rep 68:953–958, 1984]

Within the past decade, the prognosis of children with acute leukemia has greatly improved due to several factors, including further advances in multidrug therapy, the use of immunologic markers in diagnosis to more accurately classify subtypes of the disease, and better patient management and supportive care (1-4). The survival of white patients < 15 years of age diagnosed with acute lymphocytic (lymphoblastic) leukemia (ALL) and acute nonlymphocytic leukemia (ANLL) in hospitals participating in the National Cancer Institute's (NCI) End Results Group (ERG) Program increased considerably from the 1950s to the early 1970s (5). Five-year survival estimates for children diagnosed with ALL and ANLL between 1973 and 1978 are now available from the NCI's Surveillance, Epidemiology, and End Results (SEER) Program and it is apparent that the improvements in prognosis for these patients have continued into the latter part of the 1970s.

DATA AND METHODOLOGY

The analysis of trends in acute leukemia survival among white children < 15 years of age diagnosed between 1973 and 1978 was based upon data collected by nine population-based tumor registries participating in the NCI's SEER Program (6). Seven areas began submitting data in 1973: the San Francisco/Oakland metropolitan area, Connecticut, the Detroit metropolitan area, Hawaii, Iowa, New Mexico, and Utah. The Seattle/Puget Sound metropolitan area began submitting data in 1974 and the Atlanta metropolitan area began in 1975. The collection of cancer data within a defined population base allows measures of incidence, mortality, and survival to be related to the same population at risk. Survival rates based on the SEER data will not be directly compared with those based on the earlier ERG data, because different populations were covered by each series.

[1]Received June 2, 1983; revised Jan 12, 1984; accepted Jan 23, 1984.

[2]Biometric Research and Analytic Studies Section, Biometry Branch, National Cancer Institute, Bethesda, MD.

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Reprint requests to: Sandra C. Steinhorn, Biometric Research and Analytic Studies Section, Biometry Branch, National Cancer Institute, National Institutes of Health, Blair Bldg, Rm 514, Bethesda, MD 20205.

For this study, a total of 863 white children < 15 years of age diagnosed with acute leukemia in the nine SEER areas from 1973 through 1978 were selected for analysis. This figure excluded those patients diagnosed solely through a death certificate or autopsy report (three patients); those alive without follow-up, ie, alive with the diagnosis date equal to the follow-up date (eight); and those not in active follow-up (39). In the SEER Program, this implies that follow-up of vital status has not been done for these patients. The determination of cell type was based upon routine pathological review. By these criteria, only 67 black children were diagnosed with acute leukemia within this same time period, too few to permit the analysis of racial differences.

In the sample of white children, there were 645 diagnosed with ALL, 113 with acute granulocytic (myelogenous) leukemia (AGL), ten with acute monocytic leukemia, two with acute erythrocytic leukemia, and 93 with acute leukemia, NOS.[4] The male/female ratio was approximately 1.4 to 1 for patients with ALL and 1.1 to 1 for patients with AGL. ALL was diagnosed at the following ages: < 5 years (54%), 5-9 years (28%), and 10-14 years (18%). Children diagnosed with AGL between 1973 and 1978 had a slightly different age distribution: 39% were < 5 years of age, 26% were 5-9 years of age, and 35% were 10-14 years of age. The value of age, sex, and cell type as prognostic indicators of length of survival for childhood acute leukemia patients was examined for each cohort using Cox's proportional hazards model (7).

The measures used in the trends analysis were the 3- and 5-year observed survival rates. The cohort diagnosed in 1973-1975 was compared to patients diagnosed in 1976-1978 for each cell type, sex, and age at diagnosis group. The dates were chosen to allow for adequate numbers of patients in each cohort and to permit further analysis within subgroups. Differences in survival within each cohort by these three factors were also examined. The cut-off date for this study was December 1981, and thus a full 5 years of follow-up were not available for patients diagnosed in 1976-1978. Survival rates for this cohort for time intervals between 3 and 5 years from diagnosis were projected using the corresponding interval survival rates for the 1973-1975 cohort. The use of this estimation technique was based on the assumption that the survival experience of children in the later cohort from 3 to 5 years after diagnosis was at least equivalent to the survival of those in the earlier cohort. This assumption is consistent with previous analyses of survival trends (8) and appears to be a conservative procedure.

To test statistical significance, the difference between two survival rates was divided by its standard error and compared to values of the normal distribution. Survival trends between cohorts were tested using a one-tailed test of significance, whereas differences by cell type, sex, and age were tested using a two-tailed test. The survival analysis by cell type was based on those patients with ALL and AGL, because there were too few patients with acute monocytic and acute erythrocytic leukemia to permit a valid comparison. Survival for patients diagnosed with acute leukemia, NOS was also examined but discussed separately. Significance tests were not performed for comparisons with the projected 5-year survival rates of the 1976-1978 cohort.

Leukemia incidence was analyzed for all cell types combined and by major type: ALL, AGL, and all other leukemias (including acute leukemia, NOS). Children diagnosed with acute leukemia comprised approximately 94% of the total diagnosed with all types of leukemia. Over 76% of patients with acute leukemia were diagnosed with ALL and nearly 14% with AGL. Due to problems in the assignment of the correct cause of death by type for leukemias, the mortality analysis was based on the total number of leukemias (all types combined) diagnosed among children < 15 years of age, with all races and sexes combined, in the SEER areas from 1973 through 1979.

Incidence data were obtained directly from the tumor registries, whereas mortality data were provided by the National Center for Health Statistics (Health Services and Mental Health Administration, Public Health Service, US Department of Health and Human Services). To permit valid comparisons despite differences in the age distribution over geographic area and time, the age-adjusted rates were standardized by 5-year age groups to the 1970 US population < 15 years of age. Trends in the age-specific incidence rates of 5-year age groups were studied for children < 15 years of age, and for adolescents 15-19 years of age and young adults 20-24 years of age as well, to determine whether childhood leukemia mortality does, in fact, experience a time lag and is actually being delayed into older age groups as has been previously hypothesized (5).

RESULTS

Trends in Patient Survival

Improvements in survival between 1973-1975 and 1976-1978 were observed for children diagnosed with ALL in the SEER areas, but not for those with AGL. Between 1973-1975 and 1976-1978, statistically significant increases in survival were observed at 3 years for children diagnosed with ALL (from 59% to 74%). The 5-year survival rate for patients diagnosed in 1973-1975 was 49%. If we assume that children diagnosed in the SEER areas during 1976-1978 will experience at least the same survival 3-5 years from diag-

[4]NOS = not otherwise specified.

nosis as those seen in 1973-1975, then survival at 5 years from diagnosis for the later cohort may be projected at 62%.

The survival experience of white children < 15 years of age diagnosed with ALL and AGL in the nine SEER areas during the years 1973-1978 is shown in figure 1. Among children diagnosed in 1973-1975, those with ALL experienced better survival prognosis than those with AGL at intervals of 3 years (36 months) and 5 years (60 months) from diagnosis, with statistically significant differences at the 0.05 level. The standard errors for the AGL rates were between 5% and 10%. All rates quoted for ALL had standard errors < 5%. Significant differences by cell type were also noted at 3 years for patients diagnosed in 1976-1978.

Survival rates by age at diagnosis for white children with ALL in the SEER areas between 1973 and 1978 are presented in figure 2. Younger children 1-4 years of age at diagnosis had better prognosis than older children 5-14 years of age, with statistically significant differences noted at 3 and 5 years from diagnosis for the 1973-1975 cohort, and at 3 years for the 1976-1978 cohort. Both age groups showed significant increases in 3-year survival rates between 1973-1975 and 1976-1978. Survival at 5 years for the earlier cohort was 55% for the younger children and 43% for the older age group. Projected 5-year survival for patients diagnosed in 1976-1978 was 71% for those 1-4 years of age and 55% for those 5-14 years of age.

Among children < 15 years of age diagnosed with ALL in the SEER areas, the survival rates of girls exceeded those for boys at 3 years from diagnosis for both cohorts and at 5 years for those diagnosed in 1973-1975, although these differences were not statistically significant. When the combined effect of sex, age at diagnosis, and cell type on length of survival among children diagnosed with acute leukemia in the SEER areas was analyzed using Cox's proportional hazards model, sex was found to have relatively little prognostic value. Statistically significant increases in 3-year survival rates between 1973-1975 and 1976-1978 were noted both for males (56% vs 71%) and for females (63% vs 79%). For children diagnosed in 1973-1975, survival at 5 years was 54% for females and 46% for males. Projected 5-year survival for the 1976-1978 cohort was 68% for females and 58% for males.

Trends in Incidence and Mortality

The improvements in survival found among children diagnosed with ALL are reflected in the patterns of leukemia incidence and mortality observed in the SEER areas between 1973 and 1979 (fig 3). During this time period, the age-adjusted incidence rate for all childhood leukemias combined (labeled as Total Incidence in fig 3) remained relatively stable, ranging from 3.7 per 100,000 children < 15 years of age in 1973 to 3.5 per 100,000 in 1979. The incidence of ALL, which comprises > 70% of all leukemias diagnosed among SEER area children, follows this same pattern. The incidence of both AGL and all other leukemias appears to be

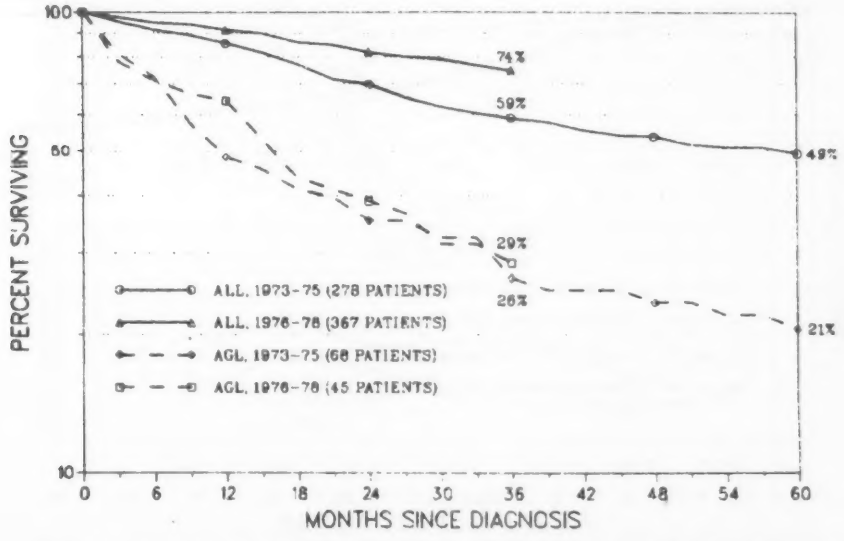

FIGURE 1.—Survival of white children < 15 yrs of age diagnosed with ALL and AGL, 9 SEER areas, 1973-1978.

trending downward, although these rates may be subject to wide variation due to the small number of cases involved in their computation. The corresponding age-adjusted total mortality rate dropped from 2.2 per 100,000 in 1973 to 1.7 per 100,000 in 1979, an average annual decrease of approximately 3%.

The age-specific incidence of leukemia among children 0-4 years of age was consistently higher than the corresponding age-specific rates for those 5-9 and 10-14 years of age between 1973 and 1979 (fig 4). Incidence rates remained fairly constant in all three age groups. The incidence of ALL and all other leukemias by age group followed the pattern for all types combined, with the highest incidence among those < 5 years of age. There appeared to be no age differences for patients with AGL. The incidence rates by type and age group were also subject to considerable variation. Age-specific mortality was higher for children 5-9 years of age throughout most of the time period, but steadily decreased approaching the rates observed for those 0-4 and 10-14 years of age in 1979.

The age-adjusted leukemia incidence and mortality rates among male children were consistently higher than the corresponding rates for females throughout the entire period, but appeared to decrease at a slightly greater rate (not shown). The incidence of ALL and all other leukemias by sex exhibited this same pattern, whereas there appeared to be no difference between males and females in the incidence of AGL from 1973 through 1979. The rates by sex were somewhat unstable throughout this period, since they were based on smaller numbers. The incidence among males fell from 4.0 per 100,000 in 1973 to 3.7 per 100,000 in 1979; among females, age-adjusted incidence was 3.4 per 100,000 in both 1973 and 1979, with minimal fluctuation during the intervening years. The mortality rate among males was 2.7 per 100,000 in 1973 decreasing to 2.0 per 100,000 in 1979; among females, the corresponding rates were 1.7 and 1.3 per 100,000, respectively.

DISCUSSION

Since the early 1960s, significantly higher survival rates have been reported among children diagnosed with ALL as opposed to those diagnosed with ANLL and, beginning in the mid-1960s, children with ALL diagnosed at 1-4 years of age had significantly higher survival than those ⩾ 5 years of age (5). Although survival rates based on the earlier ERG data could not be compared to the more recent SEER data because different populations were covered, it does appear that the improvements in prognosis among children with ALL have continued into the 1970s for those diagnosed in the SEER areas.

Although cohort to cohort improvements were observed for children with AGL (survival at 3 years was 27% for those diagnosed in 1973-1975 and 29% for those diagnosed in 1976-1978), this increase was not statistically significant. Survival at 5 years for children diagnosed in 1973-1975 was 21%; projected 5-year survival for those diagnosed in 1976-1978 was 22%.

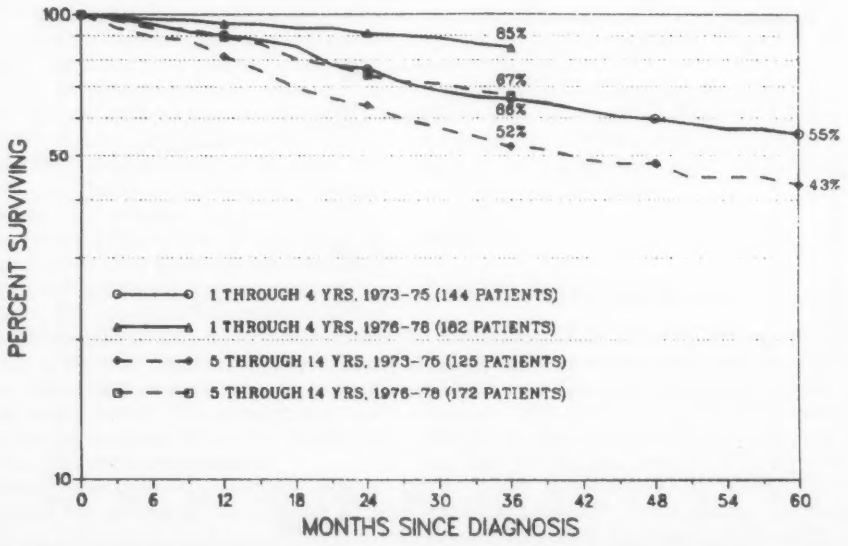

FIGURE 2.—Survival of white children < 5 and 5-14 yrs of age diagnosed with ALL, 9 SEER areas, 1973-1978.

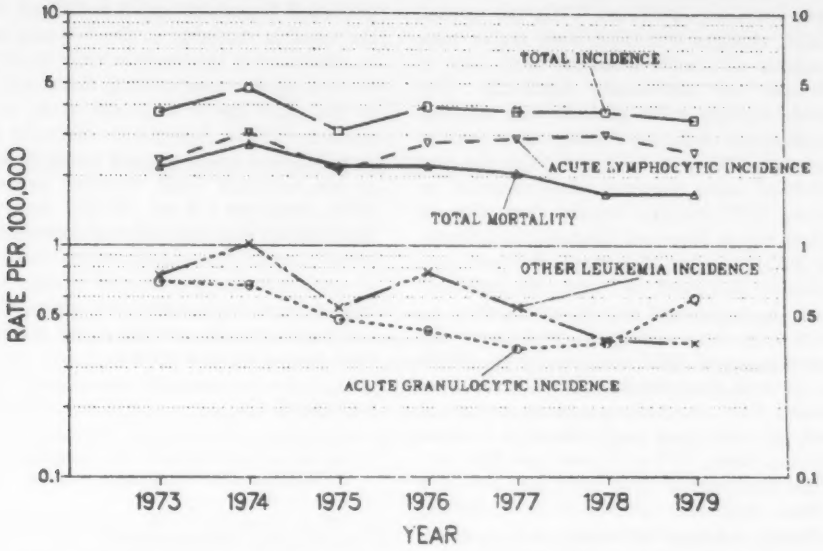

FIGURE 3.—Age-adjusted incidence and mortality rates per 100,000 population < 15 yrs of age (1970 US standard), all leukemias, 9 SEER areas, 1973–1979.

Survival rates for patients with acute leukemia, NOS were fairly close to those observed for patients with ALL. Survival was 53% at 3 years and 45% at 5 years for the earlier cohort, and 66% at 3 years for the later cohort, with standard errors between 5% and 10% for all three rates. The increase in survival between co-

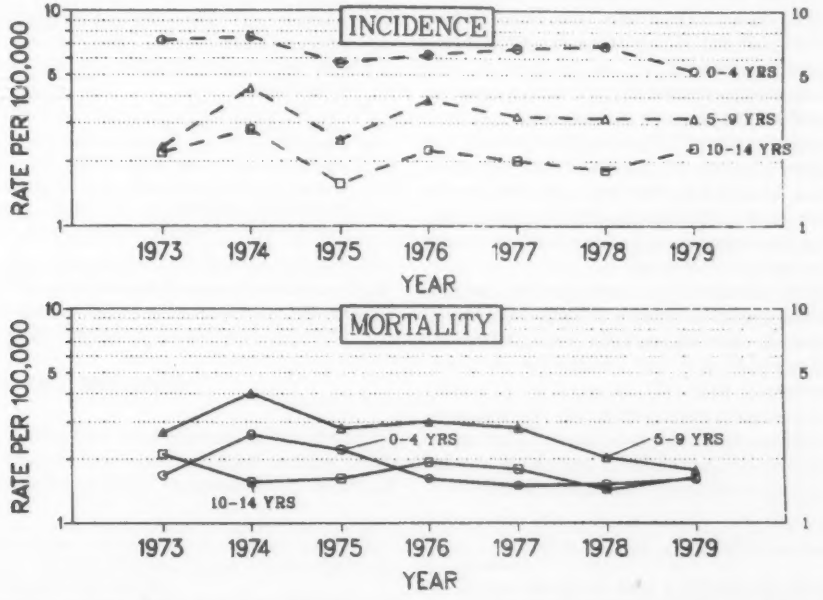

FIGURE 4.—Age-specific incidence and mortality rates per 100,000 population < 15 yrs of age by 5-yr age groups, all leukemias, 9 SEER areas, 1973–1979.

horts at 3 years was not significant. While the survival rates for SEER children with ALL were higher than those for patients with acute leukemia, NOS, none of these differences was statistically significant. This group probably contains many patients with misdiagnosed or undiagnosed ALL, particularly in the earlier years of the study. Between 1973 and 1978, the percent of childhood acute leukemia cases classified as acute leukemia, NOS dropped steadily from 17% to 4%, most likely due to improved diagnostic techniques. Birch et al (9) have noted that many childhood leukemias previously diagnosed as "stem cell leukemia" are lymphatic in origin and should be classified as ALL. Unfortunately, these cases could not be separated from the acute leukemia, NOS category for the SEER data. When survival for patients with either ALL or acute leukemia, NOS (ie, both categories combined) was analyzed, the rates were nearly the same as those reported for ALL alone: 58% at 3 years and 48% at 5 years for those diagnosed in 1973-1975, and 74% at 3 years for those diagnosed in 1976-1978. Therefore, while this change in diagnostic classification could explain some of the increases in survival among children with ALL, it does not appear to have had a major effect on the improvements observed.

Children < 1 year of age diagnosed with ALL were excluded from the analysis of age as a prognostic factor, because there were too few cases to provide reliable estimates. Although this group was not analyzed separately, it was included in the total < 15 years of age. In general, these children have been found to have poorer survival (10) and higher frequency of CNS and extramedullary involvement (11), as compared to older children. Leukemia in children < 1 year of age may also be complicated by the presence of birth defects (12). In the SEER areas between 1973 and 1978, there were 43 white children < 1 year of age diagnosed with acute leukemia (ALL plus ANLL). Survival at 3 years for this group was 31%, as compared to 71% for those diagnosed with acute leukemia at 1-4 years of age during the same time period (413 cases).

The observed decrease in the age-adjusted mortality from childhood leukemia appears to have been due to the decreases in the age-specific rates noted for patients 5-9 years of age. It is difficult to determine if the trend in mortality in the 5-9-year age group may be attributed to the improvements in the 3- and 5-year survival rates among patients diagnosed before 5 years of age, since age at diagnosis is not recorded on the death certificate. Age-specific mortality among adolescents 15-19 years of age and young adults 20-24 years of age remained fairly constant between 1973 and 1979, averaging 1.8 per 100,000 population for both age groups. This may be a good indication that leukemia mortality diagnosed among children < 15 years of age is not merely postponed to older age groups and that an increasingly higher percentage of childhood leukemia patients are now surviving to the point where their disease is under control.

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Hypersensitivity Reactions Induced by Etoposide[1]

Peter J. O'Dwyer[2,*] and Raymond B. Weiss[3,4]

Eight patients with hypersensitivity to etoposide are reported. The clinical characteristics and outcome of the patients are detailed. In four patients, treatment was given to ameliorate the symptoms. Two of these patients were successfully re-treated with etoposide after premedication with diphenhydramine and hydrocortisone. Hypersensitivity is now recognized as a form of acute toxicity occasionally occurring with this agent. The mechanism is unknown but several possibilities are discussed. [Cancer Treat Rep 68:959–961, 1984]

Recent reviews (1,2) have documented that many of the cancer chemotherapeutic agents can produce hypersensitivity reactions. Some drugs cause such reactions with frequency as high as 25% and others do so only very rarely (anecdotal cases). Many of the Type I (the most common) reactions produced by these drugs seem to be mediated by nonimmunologic mechanisms; ie, they cause release of vasoactive substances from basophils and mast cells without an intervening antibody (1,2).

The epipodophyllotoxin teniposide has caused hypersensitivity reactions (usually Type I) in 2%–11% of patients treated (1,2). Its congener, etoposide, differs in chemical structure only by the substitution of the thenylidine group on the glucopyranoside with a methyl group. Despite a close similarity in chemical structure, only a few cases of hypersensitivity reactions from etoposide have been described, and most of the clinical features of these cases are poorly documented (1,2). We report here eight cases of hypersensitivity reactions (all Type I) that have been brought to the attention of the Investigational Drug Branch of the National Cancer Institute. We describe the details of the reactions and attempt to elucidate the mechanism of their occurrence.

MATERIALS AND METHODS

The patients described were reported to the Investigational Drug Branch from January 1982 to May 1983. They were being treated with etoposide either on one of the approximately 150 protocols that have been approved by the National Cancer Institute for the investigation of etoposide or under Group C guidelines for small cell lung cancer. The total number of patients treated with this agent, from which this sample of eight patients is drawn, is not known. The reporting of these cases probably reflects enhanced awareness in the oncology community of the mechanisms for the reporting of adverse drug reactions.

RESULTS

Patient characteristics and hypersensitivity reactions are shown in table 1. Age range (17–67 years; median, 57.5) and predominance of patients with small cell lung cancer reflect the clinical activity of etoposide. No patient had a previously documented allergic diathesis or a known specific drug allergy. Only three of the eight patients had had prior exposure to etoposide; five were receiving the drug for the first time. Two patients had received other cytotoxic agents in the 24 hours prior to etoposide administration. As closely as can be ascertained, the rate of drug delivery was not excessively rapid in any patient. The dose of etoposide was $\leq$ 125 mg/m^2 in all patients. The onset of symptoms or signs was early ($<$ 10 minutes) in six patients. In one patient it occurred as the infusion was completed (after 1 hour) and in another patient it occurred 12 hours after administration. The predominant symptoms were dyspnea in five

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[2]Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]Section of Medical Oncology, Walter Reed Army Medical Center, Washington, DC.

[4]The excellent technical assistance of Ms. D. Scott, and the capable secretarial skills of Ms. M. Busits and Ms. M. L. Boyce are gratefully acknowledged.

[*]*Reprint requests to:* Peter J. O'Dwyer, MD, Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Landow Bldg, Rm 4C09, 7910 Woodmont Ave, Bethesda, MD 20814.

TABLE 1.—Patient characteristics and hypersensitivity reactions to etoposide

Patient No.	Age (yrs)	Sex	Disease*	No. of prior courses	Other drugs	Onset†	Blood pressure	Bronchospasm	Flushing	Time to recovery	Treatment	Rechallenged
1	61	M	SCLC	0	—	Early	Increase	-	+	15 mins	-	No
2	63	M	MM	0	—	Late	Decrease	+	-	—	+	No
3	56	M	SCLC	0	—	Early	Increase	+	-	Not stated	+	Yes
4	17	M	Lymphoma	0	—	Early	Increase	-	+	5 mins	-	No
5	49	F	Sarcoma	2	Dactinomycin, vinblastine	Late	—	+	-	Hrs	+	Yes
6	67	M	SCLC	4	—	Early	Increase	-	+	5 mins	-	No
7	50	F	Breast cancer	0	Doxorubicin	Early	Decrease	-	+	30 mins	-	No
8	59	F	SCLC	1	—	Early	Decrease	+	-	30 mins	+	Yes

* SCLC = small cell lung cancer; MM = multiple myeloma.
† Early = within 10 mins of starting infusion; late = > 10 mins.

patients and substernal chest discomfort in two, only one of whom had hypotension. Two patients experienced transient loss of consciousness. Other manifestations observed included hypertension in four patients, hypotension in three, bronchospasm in four, and diffuse skin flushing in four. Treatment was administered to four patients. In three of these patients, diphenhydramine, epinephrine, aminophylline, or hydrocortisone, in varying combinations, were used with rapid resolution of symptoms. In one patient the use of bronchodilators, corticosteroids, and pressor agents did not prevent a rapid clinical deterioration, and the patient died with a clinical picture and autopsy confirmation of adult respiratory distress syndrome. The role of etoposide in the death of this patient is unclear. With or without treatment, resolution of symptoms occurred in ⩽ 30 minutes in six patients; one required aminophylline for several hours. This patient was re-treated with etoposide 24 hours later while still receiving aminophylline. Bronchospasm recurred and was again resolved after several hours. Two other patients, both of whom presented with bronchospasm, were successfully re-treated after premedication with diphenhydramine and hydrocortisone.

DISCUSSION

Hypersensitivity reactions are a significant problem with many of the cytotoxic agents now in use (2). Most reactions have been Type I according to the Gell and Coombs classification (3), but the precise mechanisms for some of the reactions are unclear.

The reactions experienced by the eight patients described here are consistent with a Type I pattern of hypersensitivity. It is notable that five patients had had no prior exposure to etoposide, a phenomenon observed with other chemotherapeutic agents such as amsacrine and doxorubicin (2). There was no difference in clinical presentation or response according to the patients' prior treatment, yet two distinct clinical patterns may be seen from table 1. Patients presented with either bronchospasm or flushing, but not both. Blood pressure changes were independent of these. Patients with bronchospasm required more treatment, with slower resolution of symptoms, than those without. Immunologic testing of these patients was not performed. However, the fact that the hypersensitivity occurred on the first dose in five patients suggests that a nonimmunologic mechanism underlies some of the reactions.

The apparent greater frequency of allergic reactions to the etoposide analog teniposide has been previously mentioned (2). While studies have not been done to elucidate the reason for this apparent difference, one explanation may lie in the formulation of these agents. Polyethoxylated castor oil (Cremaphor; 2.5 g) is present in each 50-mg ampule of teniposide, while etoposide does not require this agent for solubility (4). Other drugs formulated

with Cremaphor include phytonadione (Aquamephyton) and miconazole, each of which may cause similar hypersensitivity reactions.

Another solvent, dimethylacetamide, is also used in the formulation of teniposide and amsacrine, while etoposide is formulated with polysorbate 80 (Tween 80), a surfactant with a potential role in these observed reactions.

Four of the eight patients received treatment for their reactions. All had bronchospasm, and of the three who survived, only one required bronchodilators for > 30 minutes. Each of these three patients was rechallenged with etoposide. The two patients who were premedicated with diphenhydramine and hydrocortisone had no further reactions, while the single patient receiving aminophylline alone had recurrent bronchospasm. This suggests that patients without bronchospasm require expectant treatment only, and that in circumstances in which etoposide is crucial to a patient's treatment, it might be cautiously reintroduced under antihistamine and corticosteroid coverage. However, a larger experience will be required before any recommendation can be made with confidence.

In conclusion, it is emphasized that all these reactions have been reported since January 1982, while etoposide has been in clinical use since 1971. This may indicate that there has been underreporting of this phenomenon previously.

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Vindesine, Carmustine, Doxorubicin, and Prednisone (EBAP) in Recurrent Lymphomas: A Southwest Oncology Group Study[1,2]

James K. Weick,[3] Stephen E. Jones,[4] Petre N. Grozea,[5] Carol J. Fabian,[6] and Dennis O. Dixon[7,*]

Seventy-eight patients with Hodgkin's disease and non-Hodgkin's lymphoma who had failed prior chemotherapy or radiation therapy were treated with a combination of vindesine, carmustine, doxorubicin, and prednisone (EBAP). Outpatient therapy was administered at 21-day intervals. Complete and partial responses were seen in 32 patients (41%). The response rate was higher for Hodgkin's disease (59%) than for non-Hodgkin's lymphoma (31%). The median duration of response was 31 weeks in both groups, with a median survival of all patients of 35 weeks (responders, 122 weeks; nonresponders, 16 weeks). Myelotoxicity was greater using EBAP than in the earlier reported program with vincristine, carmustine, doxorubicin, and prednisone, and in the absence of higher response rates does not support the use of vindesine over vincristine in combination programs using nitrosoureas, anthracyclines, and prednisone. [Cancer Treat Rep 68:963–967, 1984]

The Southwest Oncology Group (SWOG) has earlier reported a trial combining vincristine, carmustine, doxorubicin, and prednisone (VBAP) in patients with previously treated recurrent lymphomas (1). Responses were seen in 41% of all patients, with minimal toxicity. Median duration of remission for Hodgkin's disease and non-Hodgkin's lymphoma was 51 and 22 weeks, respectively, in 68 evaluable patients.

Phase II trials of vindesine (Eldesine) in patients with advanced lymphomas have shown activity with no clinical cross-resistance to vincristine being reported (2–7). In 1979, we decided to follow the earlier VBAP study with a trial substituting vindesine for vincristine. The first 26 cases were reported earlier by Jones et al (8), and the following represents a completed study of 78 patients.

PATIENTS AND METHODS

Patients were eligible for SWOG trial No. 7967 if they had had either a relapse after successful chemotherapy or radiation therapy, or if primary treatment regimens had failed. The total prior dose of doxorubicin could not exceed 390 mg/m^2, and no patients received prior nitrosourea compounds. Measurable tumor masses were required for evaluating response. Pathologic material was classified by the Rappaport classification for non-Hodgkin's lymphoma (9) and the Lukes-Butler classification for Hodgkin's disease (10). Survival and remission duration curves were calculated by the method of Kaplan and Meier (11), and comparisons of survival and remission duration curves between various groups of patients were made by the modified Wilcoxon test (12). Informed consent was obtained for all patients in accordance with institutional and Food and Drug Administration policies.

The pretreatment staging included: tumor measurements, cbc, electrolytes, liver function tests, ECG, bone marrow aspiration and biopsy, and chest roentgenogram.

Treatment courses were given at 21-day intervals as follows: vindesine was given iv at a dose of 2 mg/m^2 on Day 1, together with iv doses of carmustine and doxorubicin, both at 30 mg/m^2. Prednisone was given orally at 60 mg/m^2/day for 4 consecutive days. Therapy was continued until a maximum dose of 550 mg/m^2 of doxorubicin had been reached or until progressive disease was noted. At maximum doses of doxorubicin, the investigators' option was either to discontinue therapy or to continue treatment without the anthracycline. The initial doses of myelotoxic drugs were decreased by 25% with any of the following conditions: age > 70 years, serum creatinine > 2.5 mg/100 ml (decrease only of carmustine),

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[3]Cleveland Clinic Foundation, OH.

[4]University of Arizona Health Sciences Center, Tucson.

[5]Oklahoma Medical Research Foundation, Oklahoma City.

[6]University of Kansas Medical Center, Kansas City.

[7]Southwest Oncology Group Biostatistical Center, Houston, TX.

Reprint requests to: Southwest Oncology Group (SWOG-7967), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

initial granulocyte count of 1500-2500/mm^3 persisting ⩾ 6 weeks after the last therapy, and a bilirubin > 1.3 mg/100 ml. Subsequent courses were administered only after the granulocyte and platelet counts had returned to pretreatment levels. An adequate trial was considered to be two courses of therapy over a 6-week observation period, in the absence of progressive disease. Complete remission was defined as the disappearance of all clinical evidence of tumor for a minimum of 4 weeks, with no simultaneous appearance of new lesions. A partial remission was defined as a ⩾ 50% decrease in the sum of the products of the maximal perpendicular diameters of all measurable lesions.

RESULTS

Seventy-eight patients (42 men and 36 women) ranging in age from 17 to 87 years (median, 52) were treated with vindesine, carmustine, doxorubicin, and prednisone (EBAP). One additional patient was registered but never received any therapy and is not included in the evaluation. There were three early deaths 3-10 days after patients received the first course of therapy for which no postmortem examinations were performed and no conclusions about cause of death could be made; however, these patients are included in the analysis. Other patient characteristics include a performance status of 0-1 (Karnofsky, 70-100) in 26 patients, a performance status of 2 (Karnofsky, 50-60) in 39 patients, and a performance status of 3 (Karnofsky, 30-40) in an additional 13 patients. Good-risk doses, as defined above, were given to 56 patients and poor-risk doses were given to 22 patients.

All patients had received prior chemotherapy; the number of prior chemotherapy combinations averaged 1.6 per patient. Sixty patients had responded to at least one of the prior treatment regimens, either chemotherapy or radiation therapy. No prior response had been noted for 11 patients, and for seven additional patients, the data on response to prior therapy were not available to the investigator. The time from diagnosis of malignant lymphoma until EBAP was administered varied from 3 months to 16 years but averaged 4.1 years.

A total of 379 courses was administered to 78 patients, for an average of 4.9 courses per patient. The results of therapy are shown in table 1 and include 32 responders (41%) of all eligible patients. These include ten complete responders (13%) and 22 partial responders (28%). If these numbers are evaluated by tissue type, the response rate is 16 of 27 patients (59%) for Hodgkin's disease and 16 of 51 (31%) for non-Hodgkin's lymphoma. In the non-Hodgkin's lymphomas, the rate of response in diffuse histology was 30% (ten of 33 patients), whereas in nodular histology, the response rate was 33% (six of 18). Forty-seven patients had received prior doxorubicin, whereas 31 patients had not. Similar to our experience with VBAP, patient response was less likely among those

TABLE 1.—Patient characteristics for EBAP in malignant lymphoma

Characteristic	No. of patients (%)*			
	Total	CR	PR	CR + PR
Hodgkin's disease				
Lymphocyte predominance	1	1	—	1 (100)
Nodular sclerosis	17	3	7	10 (59)
Mixed cellularity	6	1	3	4 (67)
Lymphocyte depletion	1	0	0	0 —
Not otherwise specified	2	0	1	1 (50)
Total	27	5 (19)	11 (41)	16 (59)
Non-Hodgkin's lymphoma				
Diffuse				
Well-differentiated lymphocytic	3	0	0	0
Poorly differentiated lymphocytic	10	3	4	7 (70)
Mixed histiocytic-lymphocytic	2	0	1	1 (50)
Histiocytic	18	0	2	2 (11)
Total	33	3 (9)	7 (21)	10 (30)
Nodular				
Poorly differentiated lymphocytic	8	2	1	3 (38)
Mixed histiocytic-lymphocytic	5	0	2	2 (40)
Histiocytic	0	0	0	
Other	5	0	1	1 (20)
Total	18	2 (11)	4 (22)	6 (33)
Diffuse and nodular	51	5 (9)	11 (22)	16 (31)
Totals	78	10 (13)	22 (28)	32 (41)

* CR = complete response; PR = partial response.

treated previously with anthracyclines (28%) than among those who had not received prior doxorubicin (61%).

The duration of response for all patients varied from 6+ to 89+ weeks (fig 1), with a median of 31 weeks in the non-Hodgkin's lymphoma group and 31 weeks in the Hodgkin's disease group. The median survival of all patients is 35 weeks (fig 2). Responders survived a median of 122 weeks compared to 16 weeks for nonresponders. Table 2 shows the frequency of toxic effects associated with EBAP. Side effects were noted in 53% of patients (41 of 78) in this trial compared with 20% (15 of 75) in the earlier reported VBAP program. Although the incidence of gastrointestinal toxicity was identical in EBAP and VBAP programs (27% vs 25%), myelosuppression was more severe with EBAP than VBAP: two patients showed severe or life-threatening granulocytopenia and/or thrombocytopenia with EBAP. In this trial, five patients exhibited neurologic toxicity. In the absence of comparability of patients under study in this retrospective comparison with the earlier VBAP trial, no conclusion should be drawn relative to the degree of toxic effects for each program.

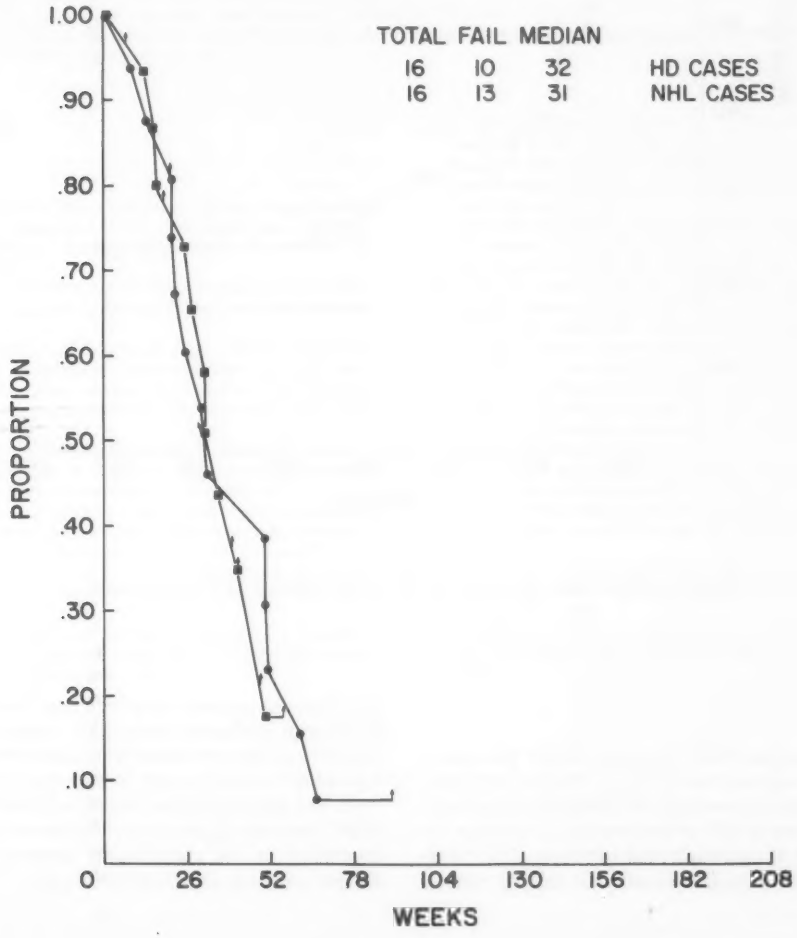

FIGURE 1.—Duration of response. HD = Hodgkin's disease; NHL = non-Hodgkin's lymphoma.

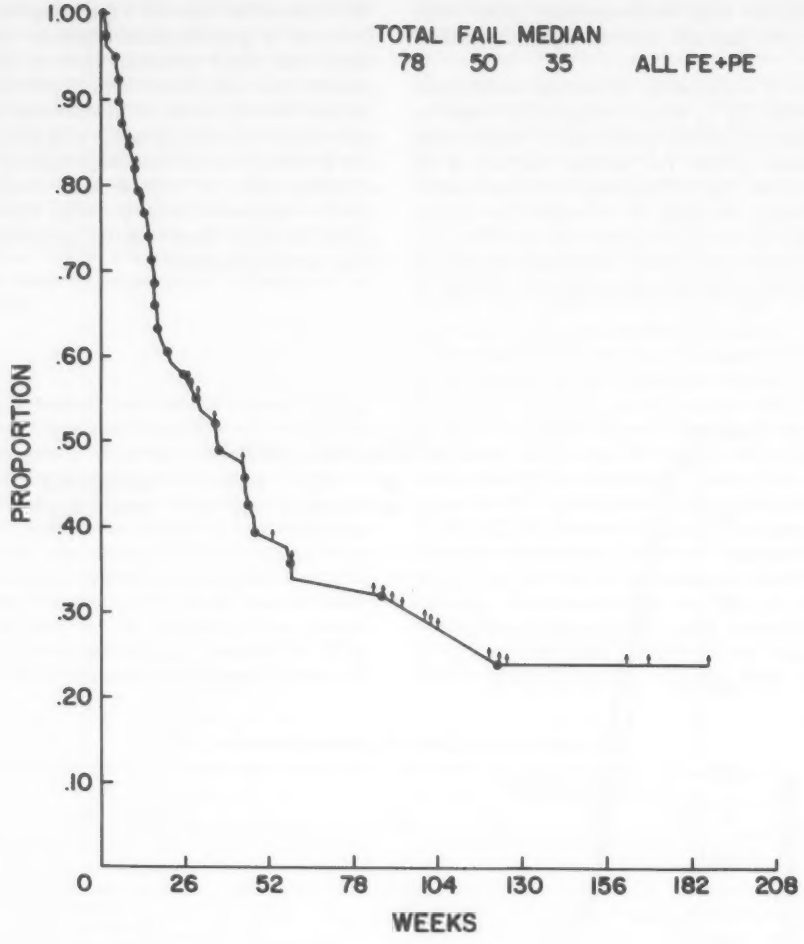

FIGURE 2.—Survival from start of treatment. FE = fully evaluable; PE = partially evaluable.

DISCUSSION

As in the original VBAP program, EBAP was also a well-tolerated regimen that produced effective palliation in both Hodgkin's disease and non-Hodgkin's lymphoma. The response rate of 41% in the two trials is identical, as is the duration of remissions and survivals. Early trials with both doxorubicin (13-15) and with the nitrosoureas (16,17) would indicate a similar range of activity for patients with malignant lymphoma. Remission duration and survival data are scanty from these early trials and it is possible that single-agent therapy may be equally beneficial. The slightly greater toxicity of EBAP compared to VBAP does not support the routine use of vindesine over vincristine in the combination program using nitrosoureas, anthracyclines, and prednisone.

TABLE 2.—Frequency of toxic effects associated with EBAP chemotherapy in 78 patients

Degree of toxicity	No. of patients with—				
	Low wbc counts*	Low platelet counts†	Gastro-intestinal toxicity‡	Neuro-pathy§	Cardiac toxicity
Mild	5	3	10	0	0
Moderate	2	1	7	4	0
Severe	5	3	4	1	0
Life-threatening	1	1	0	0	0
Fatal	0	0	0	0	0
Totals	13	8	21	5	0

*Wbc counts (cells/mm^3): mild = 3000-3999; moderate = 2000-2999; severe = 1000-1999; life-threatening = < 1000.

†Platelet counts (cells/mm^3): mild = 75,000-99,999; moderate = 50,000-74,999; severe = 25,000-49,999; life-threatening = < 25,000.

‡Gastrointestinal toxicity: mild = no bowel movement for < 2 days or 3-4 liquid stools, nausea, no vomiting; moderate = oral ulcers, able to eat, no bowel movement for 2-4 days or > 4 liquid stools; severe = unable to eat, no bowel movement for > 4 days, bloody diarrhea or vomiting > 6 times/day.

§Neuropathy: moderate = absent deep tendon reflexes, weakness and/or pain, CNS confusion; severe = weak to bedridden, convulsions or coma.

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PCNU Treatment for Recurrent Malignant Gliomas[1,2]

Victor A. Levin,[3,4,*] Kenneth J. Resser,[3,4] Lisa McGrath,[3] Pamela Vestnys,[4] Stephen Nutik,[5] and Charles B. Wilson[3,4,6]

PCNU was originally brought into clinical trials to test a quantitative structure-activity relationship (QSAR) paradigm. Based on extensive QSAR studies in L1210 (1), Lewis lung (2), intracerebral 9L (3), and intracerebral ependymoblastoma[7] tumors, we hypothesized that based on its molecular size, lipophilicity, and therapeutic index, PCNU should be superior to carmustine, lomustine, or semustine against malignant intracerebral tumors (3).

Early into phase I trials, it became evident that the potential therapeutic benefit anticipated from QSAR studies might not be realized because of higher than expected myelotoxicity (4). The differences between humans and rodents in terms of the toxicity and pharmacology of PCNU were further characterized by comparative pharmacokinetic studies in humans and rodents (5).

To test the efficacy of PCNU against recurrent supratentorial malignant gliomas and to, at least, generalize about its activity as compared to carmustine and lomustine, a population of patients with recurrent glioma was sought that had not received prior chemotherapy to more closely approximate the patient populations reported in previous studies of carmustine and lomustine (6-9). This phase II study summarizes the results of a Northern California Oncology Group (NCOG) protocol (NCOG 6G-80-1) that was designed to evaluate time to tumor progression (TTP) using criteria somewhat more stringent (10) than those used in reported carmustine and lomustine studies (*see* Discussion section).

MATERIALS AND METHODS

Patient Selection

This study began on December 16, 1980 and was closed to patient accrual on January 19, 1983. All patients with a diagnosis of glioblastoma multiforme (GM), anaplastic astrocytoma, mixed malignant glioma, gemistocytic astrocytoma, or malignant glioma presumptively diagnosed by neurodiagnostic studies were eligible. The last four subtypes constituted a group called non-glioblastoma malignant glioma (NGM) and are roughly akin to grade 3 gliomas by the Kernohan system. The subtype, malignant glioma, refers to a patient who at an earlier time had a tissue diagnosis of well-differentiated glioma, received radiation therapy, and subsequently recurred with clinical and neurodiagnostic evidence supportive of malignant transformation to a more malignant tumor. Past experience has always supported reclassification of these tumors to NGM or GM (6-9). All patients received prior radiation and some patients also received prior chemotherapy.

Treatment

Patients were treated with PCNU at a dose of 100 mg/m^2 if no previous chemotherapy had been given or 90 mg/m^2 if previous chemotherapy had been given. Treatment was repeated at 6-8-week intervals depending on myelotoxicity and was given until disease progression or for 1 year. Dose reductions were based on NCOG criteria for leukopenia and thrombocytopenia.

Evaluation

Response evaluation employed the criteria of Levin et al (10). Prior to each course of therapy, a neurological examination, computerized tomographic scan with contrast enhancement, and ^{99m}Tc-DPTA radionuclide brain scan were performed. Steroid (dexamethasone) doses

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[3]Northern California Oncology Group, Palo Alto.

[4]Brain Tumor Research Center (*V. A. Levin, P. Vestnys,* and *C. B. Wilson*) and Cancer Research Institute (*K. J. Resser*), University of California Medical School, San Francisco.

[5]Kaiser Permanente Medical Group, Redwood City, CA.

[6]We thank John Hannigan, Margaret Seager, and Shirley Hamilton for assistance in data collection and interpretation, and the doctors from the greater San Francisco Bay and Sacramento areas for referring patients for this study.

[7]Levin VA. Unpublished observations presented at the Decision Network Meeting of the Division of Cancer Treatment, 1979, and Summary of the Activity of PCNU, NSC 95466. National Cancer Institute Report, June 1978.

[*]*Reprint requests to:* Dr. Victor A. Levin, 783 HSW, University of California, San Francisco, San Francisco, CA 94143.

were kept at the lowest level consistent with good medical practice.

Evaluation and duration of response to treatment were measured from the first dose of PCNU. The criteria for response, disease progression, and stable disease were based on the comparison of sequential evaluations prior to each course of treatment and are defined as follows: *response*—definite improvement of two of three tests listed above while on a stable or decreasing steroid dose; *disease progression*—definite worsening of two of the three tests listed above while on stable or increasing steroid dose; *stable disease*—no definite worsening or improvement in two of the three tests above.

RESULTS

Table 1 summarizes the characteristics of the 46 patients entered in the study. While the main objective was to evaluate PCNU in patients with recurrent NGM tumors who had not received prior chemotherapy, some patients with other histologic types, as well as some who had received prior chemotherapy, were allowed in the study. Additionally, four patients without histologic confirmation were treated as presumed to have malignant glioma. Most patients in the study were male; about one-half had a Karnofsky performance status of 80%–100% at initiation of therapy. Of the 46 patients entered in the study, 12 had GM, 29 had NGM, four were presumed to have NGM (no surgery), and one had oligodendroglioma. No patients were ineligible. There was one "early death" (within 6–8 weeks of entry in the study). Two patients (one with NGM, no prior chemotherapy; one with oligodendroglioma) were declared not evaluable because of inadequate data. All patients except for the two deemed not evaluable are included in the analysis.

Table 2 summarizes the response to therapy for 44 evaluable patients. Of the 44 patients who were evaluated for response to therapy, 26 had NGM with no prior chemotherapy, 13 were other histologic types with no prior chemotherapy (nine with GM and four with presumed NGM), and five were patients who had received prior chemotherapy (three with NGM and two with GM). Of the NGM group who had not received prior chemotherapy, 27% (seven of 26 patients) responded to PCNU and 42% (11 of 26) stabilized (did not progress immediately) on PCNU (95% confidence limits for the true response + stable rate are 51%–81%).

The estimated median TTP for all patients with NGM who had not received prior chemotherapy was 28 weeks and, at 1 year, 23% had not progressed (fig 1). Survival was not the primary endpoint of this study, but the estimated median survival for patients with NGM who had

TABLE 1.—Patient characteristics at study entry*

	No prior chemotherapy		
	NGM	Other than NGM	Prior chemotherapy
Total	27	14	5
Age in yrs			
Mean	44.5	46.6	43.5
Range	21–76	30–66	20–60
Sex			
Male	18 (67)	12 (86)	4 (80)
Female	9 (33)	2 (14)	1 (20)
Histologic type			
GM		9 (64)	3 (60)
NGM	27 (100)		2 (40)
Presumed malignant glioma (no surgery; histology unconfirmed)		4 (28)	0 (0)
Oligodendroglioma		1 (7)	0 (0)
Karnofsky performance status			
50%–70%	13 (48)	5 (36)	2 (40)
80%–100%	13 (48)	9 (64)	3 (60)
Unavailable	1 (4)	0 (0)	0 (0)
Surgical procedure for primary diagnosis			
None	0 (0)	4 (28)	0 (0)
Biopsy only	4 (15)	0 (0)	0 (0)
Subtotal resection	23 (85)	9 (64)	5 (100)
Total resection	0 (0)	1 (7)	0 (0)
Not evaluable	1	1	0
Evaluated for response	26	13	5

* Unless otherwise specified, values = No. of patients (%).

TABLE 2.—Response to therapy

	No. of patients (%)		
	Response	Stable disease	Response + stable disease/total
Glioblastoma			
No prior chemotherapy	1 (11)	2 (22)	3/9 (33)
Prior chemotherapy	0	0	0/3 (0)
Non-glioblastoma			
No prior chemotherapy	7 (27)	11 (42)	18/26 (69)
Prior chemotherapy	0	0	0/2 (0)
Presumed glioma *			
No prior chemotherapy	0	2 (50)	2/4 (50)
Prior chemotherapy	0	0	0

* Presumed glioma = no surgery; histology unconfirmed.

not received prior chemotherapy was 53.7 weeks.

The following summarizes the experience of patients other than those with NGM with no prior chemotherapy: one of nine patients with GM with no prior chemotherapy responded; the estimated median TTP was 8.9 weeks and the estimated median survival was 16 weeks. None of five patients who had received prior chemotherapy responded or had stabilization of disease.

Table 3 summarizes the average dose (mg/m^2) of PCNU received per course of therapy for the no prior chemotherapy and prior chemotherapy groups for six courses of therapy. Table 4 summarizes the most severe toxic effects exhibited during the course of the study. As predicted by phase I studies, toxicity was cumulative and primarily limited to leukopenia and thrombocytopenia. There were three cases of severe or potentially life-threatening leukopenia (wbc count < 2000/mm^3) and 17 cases of severe or worse thrombocytopenia (platelet count < 75,000/mm^3); however, no infections or hemorrhage was noted. Only one case of severe nausea and vomiting was reported. Compared to other nitrosoureas that we have used, PCNU caused far less vomiting. There were no other nonhematologic toxic effects of note.

DISCUSSION

This phase II evaluation of PCNU demonstrated activity in patients with NGM comparable in response, TTP, and toxicity to that reported for carmustine (7-9) and lomustine (6,7). The criteria for response in the carmustine and lomustine studies differed in that they required evidence that both the clinical neurological ex-

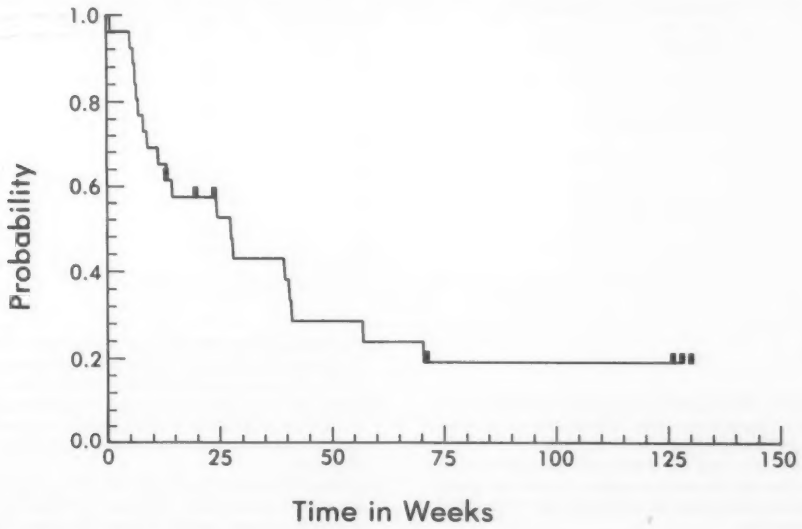

FIGURE 1.—Kaplan-Meier curve of median TTP for all patients with NGM who had not received prior chemotherapy. Ticks represent censored patients.

TABLE 3.—Average dose (mg/m^2) of PCNU received per course

Course No.	No prior chemotherapy			Prior chemotherapy
	NGM	GM	Presumed glioma	
1	96.3 (26)	96.3 (9)	96.9 (4)	70.3 (5)
2	80.1 (19)	84.1 (2)	94.7 (2)	—
3	79.2 (14)	—	—	—
4	74.2 (13)	—	—	—
5	65.5 (8)	—	—	—
6	58.2 (4)	—	—	—

* Values in parentheses = No. of patients treated.

am and radionuclide scan be improved for response and be worse for progression. Therefore, lesions in silent areas of the brain required substantial growth to produce symptoms of neurological deterioration. Because the earlier studies (6-9) did not include verification of tumor growth by computerized tomographic scan, they would somewhat overestimate TTP compared to the current study (10). Re-evaluation of reported carmustine and lomustine results indicated combined response and stable disease rates of 51% and 43%, respectively (7), with median TTP of 38 and 26 weeks. Aside from the usual criticism of historical controls, and the relationship of response to age, Karnofsky performance status, and the method of evaluating patient response, comparison to previous studies of carmustine and lomustine is difficult because of the limited numbers of patients in those studies as well as in the current PCNU study. Based on the observed activity of PCNU in this study and the fact that carmustine and lomustine are clinically available, widely used, and apparently comparable to PCNU, there appears to be no reason to make available another nitrosourea for clinical trials or to engage in large phase III trials to compare nitrosoureas in primary malignant gliomas.

TABLE 4.—Most severe toxic effects noted

Toxic effect	Grade	No. of patients
Wbc count ($\times 10^3$ cells/mm^3)		
3.0-4.0	1	9
2.0-3.0	2	6
1.5-2.0	3	1
< 1.5	4	2
Platelet count ($\times 10^3$ cells/mm^3)		
100-125	1	8
75-100	2	7
50-75	3	9
< 50	4	8
Nausea and vomiting	1	23
	2	4
	3	1
	4	0
Cutaneous: hair	1	1
	2	0
	3	0
	4	0

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Phase I Study of Diaziquone[1]

S. Frytak,* R. T. Eagan, M. M. Ames, E. T. Creagan, and W. C. Nichols[2]

Twenty-eight patients received diaziquone (AZQ) as a single iv dose ranging from 2 to 27.5 mg/m² in a phase I study. Myelosuppression was dose-limiting, tended to be cumulative, and was somewhat unpredictable in its occurrence. No pretreatment patient characteristics or chemical abnormalities were found to be consistently related to hematologic toxicity. No objective responses were noted in this study, with most patients having gastrointestinal neoplasms. A single iv injection of 22.5–27.5 mg/m² of AZQ given every 4–5 weeks would be a suitable initial schedule. Pharmacokinetic studies revealed that the plasma elimination of AZQ was best described by a two-compartment open model with rapid plasma elimination of the parent drug. [Cancer Treat Rep 68:975–978, 1984]

The aziridinylbenzoquinones constitute a class of compounds possessing physical and chemical properties (unionized, lipophilic, and low molecular weight) conducive to penetration of the central nervous system (1). Indeed, many were found to be effective against intracerebrally implanted L1210 leukemia. As a group, however, the aziridinylbenzoquinones are highly water-insoluble. One recent derivative, diaziquone (AZQ), possesses both lipid and water solubility. A comparison of 31 aziridinylbenzoquinones in two intracerebrally and in three ip implanted murine tumor systems demonstrated AZQ to be the superior analog (2). Thus, this seemed to be the most promising analog for testing in humans.

PATIENTS AND METHODS

Patients had histologic or cytologic confirmation of unresectable cancer for which no other more conventional means of therapy offered reasonable hope of cure or of significant palliation. The number and types of tumors treated during this trial are shown in table 1. Patients were excluded for the following reasons: wbc count $< 4100/mm^3$, platelet count $< 130,000/mm^3$, hemoglobin < 10 g/dl, any increase of direct-reacting bilirubin, creatinine ≥ 1.5 mg/dl, performance score (Eastern Cooperative Oncology Group [ECOG] scale) of 4, a major infectious process, recent major surgery within 30 days, previous pelvic irradiation of a significant degree (> 2500 rads to the hemipelvis or x-ray therapy to a major bone marrow-containing area within 30 days), and therapy with cytotoxic agents within the past 30 days (7 weeks for a nitrosourea, mitomycin, or dianhydrogalactitol). All patients had a complete physical examination, wbc count, hemoglobin, platelet count, 12-channel chemistry profile, serum amylase, gamma glutamyl transferase, chest x-ray, ECG, electroencephalogram, creatinine clearance, prothrombin time, short renal clearance, and urinalysis. Between treatments, patients had a wbc count, platelet count, and hemoglobin twice weekly and an SGOT and creatinine once weekly. Pulse, temperature, and blood pressure were determined prior to each chemotherapeutic treatment, and a standing and recumbent pulse and blood pressure were determined 15 minutes following drug administration and every 30 minutes for 2 hours afterwards.

Patients were entered in groups of three at each dosage level. New patients were entered at a higher dose level

TABLE 1.—No. and types of tumors treated during phase I trial of AZQ (28 patients)

Tumor types	No. of tumors
Colorectal	7
Renal	4
Lung	3
CNS	3
Skin	2
Head and neck	2
Pancreas	2
Stomach, small bowel, sarcoma, gallbladder, unknown	1 each

[1]Received Apr 29, 1983; revised Mar 14, 1984; accepted Apr 19, 1984.
[2]Oncology Department, Mayo Clinic, Rochester, MN.

Reprint requests to: Stephen Frytak, MD, Oncology Department, Mayo Clinic, 200 SW First St, Rochester, MN 55905.

only after the three patients treated at the previous level had been observed for a minimum of 3 weeks. Dosage was escalated using a modified Fibonacci scale until the first evidence of significant toxicity was noted in any of the three patients treated at a particular dosage level. When toxicity was seen, the rate of dosage escalation was not to exceed 25% of the previous dose level. When potentially dose-limiting toxicity was noted in any of the three patients at a given dosage level, three additional patients were entered at that level. No further dosage escalations were made unless the three additional patients failed to show evidence of dose-limiting toxicity. Re-treatment of any patient was based on the therapeutic response and the type, severity, duration, and reversibility of any toxic reaction. Patients showing improvement or stable disease were continued in the study, with the dose of subsequent treatments being determined by the toxicity noted following the previous therapy. Any patient showing objective progression of disease or clinical deterioration was not re-treated.

Drug Preparation, Dose, and Schedule of Treatment

AZQ was dissolved in 0.5 ml of sterile *N,N*-dimethylacetamide. This solution was further diluted with 9.5 ml of sterile 0.01 M, pH 6.5, phosphate buffer. The resulting mixture was then added to a solution of 250 ml of normal saline and given iv over a 10–20-minute period. Doses of AZQ were given in the following order: 1, 2, 5, 15, 20, 22.5, and 27.5 mg/m^2. One patient also received a single 25.5-mg/m^2 dose. Treatment was to be repeated at 4-week intervals provided the patient's clinical status and toxicity experience were acceptable.

Pharmacokinetic Studies

Venous blood samples were obtained prior to drug administration and afterwards at multiple times over a 3- to 4-hour period. Blood samples were collected into evacuated heparin-containing tubes. Plasma was separated after low-speed centrifugation and stored at -70°C until analysis. Urine samples were collected and combined for 24 hours following drug administration. Aliquots of urine were stored at -70°C until analysis.

AZQ was assayed by a modification of the high-performance liquid chromatographic (HPLC) procedure described by Kelley and Chong (3). Briefly, AZQ was extracted from plasma and urine samples (1 ml) with 5 ml of chloroform following addition of 0.5 ml of phosphate buffer (0.1 M, pH 6.5) and 50 ng of internal standard (2,5-diammino-3, 6-dichloro-1,4-benzoquinone, 50 µl of a 1-µg/ml 10% acetonitrile/water solution). Samples were shaken on a mechanical shaker for 20 minutes. Following centrifugation at low speed (1000 × *g*, 20 minutes), the organic layer was removed and concentrated under a stream of nitrogen. The residue was taken up in 50–100-µl 10% acetonitrile/water for HPLC analysis. Aliquots were analyzed on a Hewlett-Packard 1084B HPLC system equipped with a Waters Model 440 ultraviolet absorbance detector (340-nm filter). Chromatography was accomplished with a Waters 5-µm C_{18} reverse phase column with acetonitrile/water gradient elution. A guard column containing Whatman Pellicular C_{18} packing was placed in line ahead of the analytical column. The gradient elution was programmed for a linear increase of acetonitrile concentration from 10% to 30% during the first 10 minutes of the run, followed by a linear increase to 100% acetonitrile from 10 minutes to 13 minutes for column washing. Under these conditions, the internal standard eluted at approximately 6.7 minutes and AZQ eluted at approximately 9.6 minutes. Standard curves were prepared by adding known amounts of AZQ (10–500 ng) and the internal standard (50 ng) to 1-ml aliquots of thawed human plasma.

Analysis of pharmacokinetic data was conducted using the NONLIN least-squares regression analysis program (4) on a CDC Cyber 170-720 computer with interactive graphic analysis. Pharmacokinetic parameters were calculated with allowance for infusion times (3–18 minutes). The biexponential decline in plasma concentrations of AZQ was fitted to the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$, with a weighting factor of 1/Y. C is the plasma concentration of AZQ at time t after administration of AZQ; A and B are the intercepts at t = 0, and α and β are the fast and slow disposition rate constants.

RESULTS

Toxicity

Twenty-eight patients were entered in the study and received at least one course of chemotherapy. Dose-limiting toxicity was myelosuppression. Table 2 shows the number of courses producing significant hematologic toxicity (wbc count < 3000/mm^3 and platelet count < 100,000/mm^3) and the dosages at which these were encountered.

There was no significant myelosuppression at doses < 15 mg/m^2. Both patients experiencing myelosuppression at the 15-mg/m^2 dose had received prior treatment with both chemotherapy (5-FU-based regimens) and irradiation. Both patients had good pretreatment performance scores (ECOG 1 and 2, respectively). One of these patients was re-treated with AZQ at a reduced dose of 10 mg/m^2 and demonstrated a leukocyte count nadir of 2500/mm^3. There was a 6-week delay in recovering from the myelosuppression.

At the 22.5-mg/m^2 level, three patients demonstrated myelosuppression by our standards. One patient (ECOG performance score, 0) had a wbc count nadir of 1200/mm^3 and a platelet count nadir of 38,000/mm^3.

Three of five patients treated at the 27.5-mg/m^2 dose

TABLE 2.—Hematologic toxicity of AZQ*

	15 mg/m² (9 patients/9 courses)	20 mg/m² (5 patients/9 courses)	22.5 mg/m² (8 patients/8 courses)	25 mg/m² (1 patient/1 course)	27.5 mg/m² (5 patients/7 courses)
Wbc count (cells/mm³)					
2000-2999	2	1	1	1	2
1000-1999	0	1	1	0	1
Platelet count (cells/mm³)					
75,000-99,999	0	0	1	0	2
50,000-74,999	0	0	0	0	0
25,000-49,999	1	0	1	0	0
< 25,000	0	0	0	0	1

* Wbc count < 3000 cells/mm³; platelet count < 100,000 cells/mm³.

level showed significant myelosuppression. One patient, a 28-year-old female with a grade 3 liposarcoma, heavily pretreated but with an ECOG performance score of 1, developed a life-threatening thrombocytopenia (platelet count 5000/mm³) following initial treatment with AZQ at the 27.5-mg/m² dose. In addition, her wbc count dropped to 1100/mm³. This patient recovered with supportive care measures. The leukocyte and platelet count nadirs were on Days 19 and 16, respectively. The other two patients who developed myelosuppression at the 27.5-mg/m² level had received multiple courses. Following a third course at the 27.5-mg/m² dose level, one patient developed moderate myelosuppression (wbc count 2400/mm³ and platelet count 93,000/mm³), with subsequent hematologic recovery at 7 weeks. The other patient had no myelosuppression with a dose of 20 mg/m² but developed a wbc count of 2000/mm³ and a platelet count of 75,000/mm³ after treatment at the 27.5-mg/m² level.

The largest number of courses received by any patient in the study was eight. Doses ranged from 2 to 25 mg/m². Following the 25-mg/m² treatment, the patient developed leukopenia (wbc count 2700/mm³) and an 8-week delay in hematologic recovery.

Nausea and vomiting was noted occasionally at various dosage levels, but this was not of major consequence and was usually controlled with phenothiazines at standard dosages.

Therapeutic Responses

There was no objective evidence of a therapeutic response in any of the patients treated. One patient with a grade 2 oligodendroglioma who had failed radiation therapy and chemotherapy, including a nitrosourea, had stable disease for 9 months.

Pharmacologic Studies

In our experiments, the HPLC assay did not allow quantification of plasma concentrations of AZQ at doses < 15 mg/m². In addition, AZQ was not detected in the plasma of two patients who received 15 mg/m², in one patient who received 20 mg/m², and in one patient who received 22.5 mg/m². Standard curves were carried out for each assay, and there were no indications from standard curve data of assay problems for patients in whom no plasma AZQ was detected. Plasma time-concentration curves for one patient at doses of 20, 22.5, and 27.5 mg/m² are shown in figure 1. Plasma data for all patients except two (*see* below) were best described by a two-compartment open model, with a mean distributive phase half-life (α-half-life) of 3 minutes (range, 2-6) and a mean plasma elimination phase half-life (β-half-life) of 34 minutes (range, 25-63). Mean total-body clearance and apparent volume of distribution in steady state were 453 ml/minute/m² (range, 251-885) and 28.6 L/m² (range, 5.8-47.9), respectively. Plasma elimination data for two patients who received 15 or 20 mg/m² (one each) and had low plasma concentrations of AZQ were better described by a one-compartment model with plasma half-life values of 15-30 minutes. No further analysis of these data was carried out. Twenty-four hour urinary recovery of AZQ was < 1% for all patients.

DISCUSSION

On a 1-day schedule of AZQ, myelosuppression was the dose-limiting toxic effect. In general, patients having prior chemotherapy had the most significant myelosuppression, although there was considerable unpredictability in its occurrence. Some patients heavily pretreated had no significant myelosuppression at a particular dosage level, while others with less prior chemotherapy or having had no chemotherapy previously developed myelosuppression. Factors such as a patient's performance score, tumor type, and hepatic or renal function did not show a strong correlation with myelosuppression following treatment with AZQ.

Because of its interesting chemical properties and a relative lack of gastrointestinal toxicity, further testing of AZQ in humans seems justified. Considering the unpredictable nature of its hematologic toxicity (potentially life-threatening), its distinct tendency for cumulative myelosuppression, and the delayed recovery time in

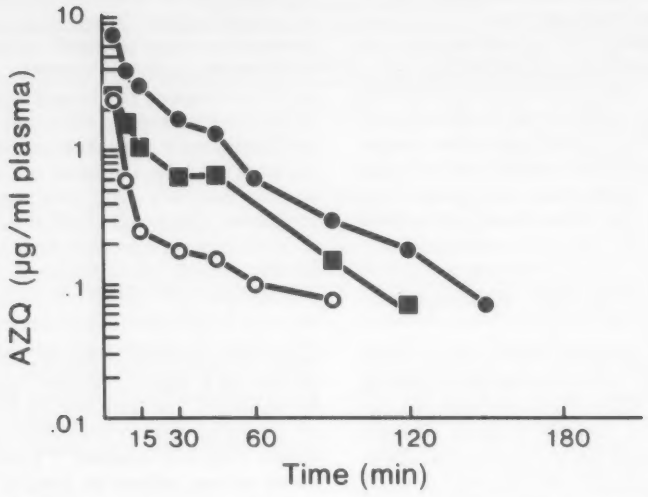

FIGURE 1.—Plasma time-concentration curves for a patient treated with AZQ at doses of 20 (○), 22.5 (●), and 27.5 (■) mg/m^2.

those patients experiencing myelosuppression, a conservative dosage schedule should be utilized for further studies. Based on our experience, a dose of 22.5 mg/m^2 would seem reasonable for all patients. In those patients without prior treatment and a good performance score, a dose of 27.5 mg/m^2 could be utilized.

Pharmacologic Studies

Pharmacologic studies showed that AZQ was eliminated from plasma by a two-compartment open model with rapid plasma elimination of parent drug. Results of our pharmacologic studies are similar to those reported by Griffin et al (5) and by Schilsky et al (6). Data from these two studies were best described by two-compartment open models with similar pharmacologic parameters to those obtained in our studies, ie, rapid plasma elimination. Rapid plasma clearance and urinary recovery of < 1% parent drug are suggestive of rapid metabolism or drug binding to blood or tissue constituents. Studies with radiolabeled AZQ suggest rapid alkylation of hepatic microsomal proteins in an NADPH-independent manner (M. M. Ames, unpublished observations). Thus, aziridine moieties of AZQ may rapidly bind to macromolecule nucleophiles, and at least partially explain the rapid plasma disappearance of AZQ.

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Phase I Trial of Vinzolidine[1,2]

Daniel R. Budman,* Philip Schulman, Mary Marks, Vincent Vinciguerra, Lora Weiselberg, Willi Kreis, and Thomas J. Degnan[3]

Vinzolidine is a new, orally active, semisynthetic vinca alkaloid which shows broad antitumor activity against murine tumor test systems. This phase I study established a 1 day every 2 week schedule of 35 mg/m² in good-risk patients and of 30 mg/m² in poor-risk patients. Maximal tolerated dose was 45 mg/m² with severe neutropenia, syndrome of inappropriate antidiuretic hormone, and paralytic ileus. Significant antitumor responses were seen in two patients with lymphoma and in one with squamous cell cancer of the lung. [Cancer Treat Rep 68:979–982, 1984]

The vinca alkaloids represent a class of nonmutagenic anticancer drugs with a broad spectrum of activity against human tumors. In an attempt to allow oral absorption of a vinca alkaloid, vinzolidine (VZL), a semisynthetic derivative of vinblastine, was synthesized. This agent (fig 1) incorporates a heterocyclic ring structure at carbon 3 and beta-chloroethyl side chain. These changes make the vinca alkaloid sterically more rigid and lipophilic in areas of the presumptive binding site of the molecule to receptor sites.

In preclinical testing, VZL was not found to be mutagenic in the Ames or activated Ames assays. In animal tumor screens, VZL was more active than vinblastine or vincristine against B16 melanoma, with increased therapeutic effect on an intermittent high-dose schedule. VZL was also active against P1534J leukemia, Gardner lymphosarcoma, GC3HED lymphosarcoma, 755 adenocarcinoma, C3H mammary carcinoma, and CA115 carcinoma. VZL was inactive against Ridgway osteogenic sarcoma, Lewis lung carcinoma, and L1210 leukemia (1). Bioavailability of VZL in rhesus monkeys was approximately 2½ times that of vinblastine when given orally (1). Preclinical animal toxicology demonstrated the lowest toxic dose to be 6 mg/m² orally twice weekly in the dog, with an LD50 of 104 mg/m² in the rat.

This phase I trial was designed to evaluate an every 2 week oral dosing schedule of VZL in man. The initial starting dosage of this trial was based on early weekly phase I data at the Lilly Clinic, suggesting minimal toxicity in man at 25 mg/m² (2). Since naturally occurring vinca alkaloids have a narrow margin of efficacy to toxicity, the dosage escalation was more modest than a traditional Fibonacci scheme. Preliminary findings were previously reported (3).

MATERIALS AND METHODS

Patients were selected by the following criteria: > 21 years of age; histologically documented inoperable or recurrent carcinoma or lymphoma refractory to other therapy; failure of a 1-month trial of "standard therapy" where one exists; Cancer and Leukemia Group B (CALGB) performance status (PS) ⩽ 2 (ambulatory) or 3 (expected survival > 6 weeks); and total bilirubin < 1.5 mg/100 ml, serum creatinine ⩽ 2.0 mg/dl, wbc count ⩽ 3500/μl, and platelet count ⩾ 100,000/μl.

All effects of prior therapy had to resolve a minimum of 21 days after radiation or chemotherapy, with a minimum of 42 days after mitomycin or nitrosourea treatment.

Informed consent was obtained according to institutional guidelines.

This trial was designed to evaluate both acute and chronic toxicity. VZL was obtained from Eli Lilly and Co, Indianapolis, IN, as an investigational drug. The drug was packaged as 5-, 10- or 20-mg capsules. All patients received capsules of VZL which were kept frozen at -10°C prior to use and ingested frozen on an empty stomach with water. The drug was divided into three

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[2]Supported in part by a grant from Eli Lilly and Co, and The Don Monti Memorial Foundation.
[3]Department of Medicine, North Shore University Hospital and Cornell University Medical College, Manhasset, NY.
* *Reprint requests to:* Daniel R. Budman, MD, Department of Medicine, North Shore University Hospital, 300 Community Dr, Manhasset, NY 11030.

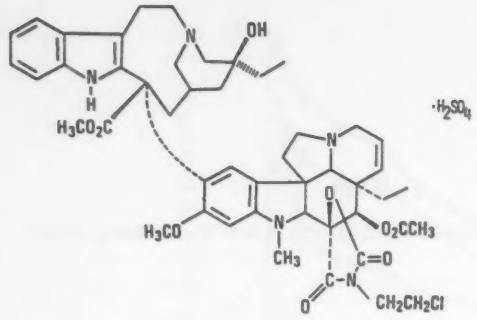

FIGURE 1.—Structure of VZL.

equal portions taken approximately 6-8 hours apart and repeated at 2-week intervals.

Patients were assigned to one of the following dose levels in sequence: 25, 30, 35, or 45 mg/m^2. A minimum of three patients were entered at each level. Once a patient was assigned to a dose level, no further dose escalation was allowed. In the presence of international grade 3 or 4 toxicity (4), subsequent dosages at that dose level were reduced by 20%, if the patient remained in the study.

All patients were evaluated for acute toxicity every third day including hemoglobin, wbc count with differential, and platelet count. At 2-week intervals, tumor measurements, serum electrolytes, glucose, BUN, creatinine, transaminase, uric acid, bilirubin, total protein and albumin, calcium, and phosphorus were determined.

In the absence of intolerable toxicity or progressive disease, the patients were continued in the study for a minimum of 1 month. Patients with rapidly progressive disease were removed sooner.

RESULTS

Twenty-two patients were entered in this trial (table 1). The average age was 61 years (range, 46-73) and the average CALGB PS was 1 (range, 0-2). Nineteen patients had measurable disease. Fourteen patients had had prior chemotherapy. Only three patients had had chemotherapy and irradiation, while one had had prior radiation therapy only. Seven patients had had prior exposure to diaziquone, mitomycin, and/or lomustine.

Five patients were entered at a dose of 25 mg/m^2. Toxicity at this level was mild (table 2) and consisted of mild nausea/vomiting and/or diarrhea which usually occurred after the second divided drug dose. One patient who required emergency radiation therapy for esophageal obstruction during the observation period developed thrombocytopenia (58,000 cells/μl). This patient had had prior lomustine, diaziquone, and mitomycin. Five patients received 30 mg/m^2, with an additional four patients receiving initial higher doses which were decreased to this level. Malaise was the most significant toxic effect.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	22
Evaluable	22
Median age in yrs (range): 61 (46-73)	
Median CALGB PS (range): 1 (0-2)	
Sex (male/female)	12/10
Prior therapy	
None	4
Chemotherapy only	
1-4 drugs	6
⩾ 5 drugs	8
Chemotherapy and radiation therapy	3
Radiation therapy only	1
Diagnosis	
Colon cancer	3
Gastric cancer	1
Non-small cell lung cancer	9
Lymphoma	4
Hypernephroma	2
Ovarian cancer	3

Nine patients were entered at a dose of 35 mg/m^2. Leukopenia manifested as reversible neutropenia was noted in four patients with grade 2-3 toxicity (total wbc count range, 1000-2000/mm^3) (table 3). Seven patients had gastrointestinal side effects. A minor lassitude syndrome occurring 12-24 hours after VZL and lasting several days was also noted at this dosage level.

Three patients were entered at a dose of 45 mg/m^2, which was the maximal tolerated dose in this study. All three patients had life-threatening leukopenia, two had transient inappropriate antidiuretic hormone syndrome, and two had severe but transient paralytic ileus lasting several days. All side effects were reversible.

The major toxic effects of > 30 mg/m^2 were dose-related reversible neutropenia (table 3), with a nadir at 11 ± 3 days, mild nausea/vomiting, and intermittent diarrhea. Thrombocytopenia occurred in 4.5% of the patients, with transient eosinophilia in 27%. Initial PS of the patient was compared to the presence or absence of grade 2 toxicity or more for VZL doses at 30 or 35 mg/m^2 (one level below the maximal tolerated dose). Ninety percent of the patients with a PS of 0-1 had less than grade 2 toxicity, while 83% of the patients with a PS of 2 had grade 2+ or greater toxicity.

Antitumor responses were seen within 2 weeks of start of therapy. One patient with stage IV refractory mycosis fungoides had a complete remission (after one course of chemotherapy) lasting 28 weeks; a patient with nodular poorly differentiated lymphoma responded after one cycle of treatment for 6 weeks as a partial responder and for 8 weeks as a complete responder.

TABLE 2.—Dose level and toxicity

Dose level (mg/m^2)	No. of patients	No. of doses given	Toxicity		
			Grade	Effect	No. of patients
20	1*	1	1+	Diarrhea	1
25	5	6	1+	Nausea/vomiting	1
			2+	Thrombocytopenia	1
			3+	Malaise	1
30	9†	42	1+	Diarrhea	1
			1+	Leukopenia, eosinophilia	1
			1+	Nausea/vomiting	2
			3+	Malaise	1
35	9	57	1+	Diarrhea	4
			1+	Mucositis	1
			1+	Nausea/vomiting	3
			2+	Leukopenia	1
			3+	Leukopenia, eosinophilia	4
45	3	3		Inappropriate antidiuretic hormone	2
			1+	Nausea/vomiting	1
			4+	Leukopenia	3
			4+	Paralytic ileus	2

* Patient de-escalated from higher dosage level.
†4 patients de-escalated from a higher dosage level because of grade 3–4 leukopenia.

One patient with non-small cell lung carcinoma remains a partial responder for 39+ weeks and continues to receive the drug. The patient with mycosis fungoides had failed 11 drugs including vincristine and vinblastine. The patient with nodular lymphoma had failed five drugs including vincristine.

DISCUSSION

VZL is a unique semisynthetic vinca alkaloid with definite antitumor activity in man as noted in this trial. Antitumor responses were seen in patients refractory to vincristine and vinblastine. In vitro, VZL was noted to show dose-dependency and lack of resistance with vinblastine (5); schedule-dependency was also noted (6). Oral availability in man (2) makes this drug attractive for further study.

Initial pharmacologic studies in man suggested a long serum half-life on the basis of a vinca-specific radioimmunoassay (2). The every other week dosage schedule was chosen in an attempt to minimize potential toxicity. This every 2 week schedule has minimized the peripheral neuropathy seen in a weekly dosing schedule (6), although lassitude remains a side effect.

The major side effect of this drug was reversible neutropenia which was dose-related but occurred in only 29% of courses at doses of ≥ 30 mg/m^2. This nonhomogeneity may represent inconsistent absorption of VZL by the oral route.

The maximal tolerated dose of 45 mg/m^2 resulted in inappropriate antidiuretic hormone syndrome, paralytic ileus, and severe leukopenia. On the basis of this phase I study, suggested phase II starting doses would be 35 mg/m^2 every 2 weeks for good-risk, untreated patients and 30 mg/m^2 for poor-risk patients.

TABLE 3.—Hematologic toxicity as a function of dose

Dose level (mg/m^2)	No. of doses given	Total wbc count ($\times 10^3$ cells/mm^3)		Neutrophil count ($\times 10^3$ cells/mm^3)	
		Nadir range	Median	Nadir range	Median
25	7	No toxicity		No toxicity	
30	36*	2.9–4.7	3.8	1.6–2.9	2.2
35	53†	1.1–4.6	3.1	1.2–2.3	1.7
45	3	0.6–1.3	0.9	0.12–0.96	0.55

* Only 4 doses had nadirs < 4.0.
†Only 12 doses had nadirs < 4.0.

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Phase II Study of Sequential Methotrexate and 5-FU Plus Mitomycin and Leucovorin in Patients With Disseminated Large Bowel Cancer: A Northern California Oncology Group Study[1,2]

Robert J. Ignoffo,[3,*] Michael A. Friedman,[3] Melanie Gribble,[4] John Hannigan,[4] Ralph Reynolds,[5] Kou-Ping Yu,[6] Steven Schiff,[7] and James E. Congdon[8,9]

Sequential methotrexate (MTX) and 5-FU plus leucovorin and mitomycin (MMC) was given to 52 patients with disseminated, measurable colorectal cancer. Complete and partial responses were seen in 19 of 49 (39%) evaluable patients. Nine additional patients achieved a minimal response. Median overall survival was 8.8 months, while that for patients objectively responding was 13.6 months. This study utilized a MTX exposure period of 12 hours prior to 5-FU. In comparison to other trials utilizing sequential MTX and 5-FU, the addition of MMC to the regimen may improve the response rate but increases the incidence of adverse effects, particularly myelosuppression and renal toxicity. This phase II trial suggests that sequential MTX and 5-FU plus leucovorin and MMC is an active combination therapy and warrants further comparative trial in patients with large bowel cancer. [Cancer Treat Rep 68:983–988, 1984]

Because advanced colorectal carcinoma responds poorly to single-agent 5-FU, studies have recently been started to investigate ways to modulate the effect of 5-FU in this disease. When methotrexate (MTX) precedes 5-FU, synergy is reported in both cell culture (1) and animal tumor (2,3) and appears to be optimal when MTX exposure is maintained for 24 hours (4,5). When 5-FU precedes MTX exposure, no synergy is observed and, in fact, is antagonistic (2). Two biochemical mechanisms have been proposed for the synergistic effect of sequential MTX and 5-FU. Bertino et al (2) and Fernandes and Bertino (6) postulated that pretreatment with MTX increases the binding of the active 5-FU moiety, 5-FdUMP, to MTX and thymidylate synthetase, thereby enhancing inhibition of DNA synthesis. Cadman et al (7) have shown that a different mechanism may be operational in which de novo purine synthesis is inhibited leading to phosphoribosyl pyrophosphate accumulation. Since phosphoribosyl pyrophosphate promotes the conversion of 5-FU to its active form, 5-FU triphosphate (5-FUTP), uptake into RNA is enhanced.

Reduced folates may also play a role in enhancing the activity of MTX and 5-FU regimens. Ullman et al (8) have shown that 5-FUdR cytotoxicity is enhanced in the presence of reduced folate cofactors. Since leucovorin is a ready source of reduced folate, it may enhance the cytotoxic activity of 5-FU if given concurrently. Thus, leucovorin may be a useful adjunct to MTX and 5-FU regimens, even for low doses of MTX.

Because of the encouraging biochemical data generated by Cadman et al (1) and by Bertino et al (2), we initially piloted a trial (Northern California Oncology Group [NCOG] 3C81-X) of sequential moderate-dose MTX (250 mg/m^2 over 3 hours) followed by 5-FU (1.0 g/m^2 by iv infusion starting at Hour 2 of the MTX infusion and given over 24 hours on Day 1 and 350 mg/m^2 by iv bolus on Days 2–4) and leucovorin rescue. However, the response rate was disappointing, with only two of 12 previously

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[3]Cancer Research Institute, Division of Clinical Pharmacy, School of Pharmacy (*R. J. Ignoffo*), and Department of Medicine (*M. A. Friedman*), University of California, San Francisco.

[4]Northern California Oncology Group, Palo Alto.

[5]David Grant Medical Center, Travis Air Force Base, Fairfield, CA.

[6]Kaiser Foundation Hospital, Santa Clara, CA.

[7]Northern Nevada Cancer Council, Reno.

[8]Naval Regional Medical Center, Oakland, CA.

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[*]*Reprint requests to:* Dr. Robert J. Ignoffo, S-926, University of California at San Francisco, San Francisco, CA 94143.

untreated patients achieving a partial remission (9). In addition, toxicity was moderately severe and the therapy required hospitalization for drug administration.

RATIONALE

Our objective was to develop a regimen which would be more effective than 5-FU alone but would also demonstrate MTX and 5-FU synergy. Since combination regimens for bowel cancer often contain an alkylating agent, we felt that the addition of mitomycin (MMC) to MTX and 5-FU might improve our results. Moertel (10) has shown that MMC has a 15% objective response rate in advanced large bowel cancer. Consequently, we designed this trial as an outpatient program and report here the results of this cooperative group phase II study of sequential MTX and 5-FU plus MMC and leucovorin rescue in advanced colorectal cancer.

PATIENTS AND METHODS

Fifty-two patients with histologically documented adenocarcinoma of the large bowel were entered with consent in this study from October 1980 to June 1981. The following eligibility criteria were met: expected survival of $\geq$ 60 days; a Karnofsky performance status (KPS) of $\geq$ 40%; adequate hepatic function (bilirubin $\leq$ 2 mg/100 ml, SGOT $\leq$ 3 $\times$ normal); adequate renal function (BUN or creatinine $<$ 50 and 2 mg/100 ml, respectively); adequate bone marrow function (wbc count $\geq$ 4000/mm^3 and platelet count $\geq$ 100,000/mm^3); and age between $\geq$ 16 and $\leq$ 85 years. Conditions of ineligibility included severe malnutrition or active second malignancy. Patients receiving brief prior therapy with 5-FU were allowed if $<$ 5-g/m^2 total dose had been given. Patients previously treated with MMC were not eligible.

The mean age of patients entered was 57.5 years (range, 23–75). The mean KPS was 84% (range, 40%–100%). Thirty-one percent of the patients had received prior pelvic radiation therapy and 15% had received minimal prior 5-FU chemotherapy. The predominant sites of disease at entry were lung (18 patients, 35%) and liver (37 patients, 71%). Other areas of involvement included peritoneum (6%), pelvis (21%), bone (12%), nodes (6%), skin (4%), ascites (2%), and brain (2%). No patients received concurrent irradiation to the site of evaluable disease.

The chemotherapy protocol consisted of MTX in oral tablets (2.5 mg) supplied by Lederle Laboratories and given at a dose of 30 mg/m^2 the night before and again the morning of clinic (60-mg/m^2 total dose). The MTX pre-exposure was 12 hours. 5-FU was given by iv push at a dose of 500 mg/m^2 on Day 2. Leucovorin was given on Day 2 at a dose of 20 mg/m^2 (nearest 5-mg oral tablet dose) every 6 hours for four doses and was started 12 hours after the second dose of MTX. MTX, 5-FU, and leucovorin were given weekly if bone marrow suppression or gastrointestinal toxicity was mild. MMC was given at a dose of 10 mg/m^2 by iv push every 8 weeks. All patients were instructed to drink 1 L of fluid prior to and during the MTX therapy. Treatment was administered weekly with MTX, 5-FU, and leucovorin if the leukocyte count was $>$ 3500/mm^3, if the platelet count was $>$ 100,000/mm^3, and if the creatinine clearance was $>$ 50 ml/minute.

Response criteria used by the group were defined as: *complete response (CR)*—the complete disappearance of all detectable disease for $\geq$ 1 month; *partial response (PR)*—a $>$ 50% decrease in the product of the longest perpendicular diameters of a clearly measurable lesion with no increase in any other lesions or new areas of malignant disease (if metastatic disease was not clearly measurable by physical or radiologic means, a $>$ 50% decrease in carcinoembryonic antigen [CEA] must be achieved for at least 1 month without progressive disease or new disease at any site); *minimal response*—between 25% and 50% tumor reduction as defined by PR for 1 month; *stable disease*—no change for 1 month; and *disease progression*—a $\geq$ 25% increase in tumor volume. Response was assessed by physical measurement of evaluable lesions on physical examination, x-ray or scan, changes in liver function tests, and CEA. The CEA titer was used as a criterion for response only in a selected group, since the value of CEA in demonstrating disease regression has been well-documented (11,12). Survival was determined starting from the day of initial chemotherapy. Toxicity was graded with each treatment according to NCOG criteria.

RESULTS

Forty-nine of the 52 patients are presented for response and toxicity analysis. One patient was not evaluable because of inadequate therapy and two were declared ineligible because of substantial prior treatment (MMC, 5-FU $>$ 5 g/m^2). Five patients had early deaths within 6 weeks of entry, one of which was attributable to drug toxicity. All five early deaths occurred among the last 27 patients entered in the study and were included in the response analysis. One CR (2%) (in a patient with liver-predominant disease), 15 PRs (32%), and three CEA responders were observed, for a total response rate of 39%. Nine minimal responses (18%) were also observed, and 12 patients (25%) had stable disease. Median duration of survival was 8.8 months. Median survival was 13.6 months for responders and 7.3 months for nonresponders ($P < 0.02$). Performance status, as in previously reported studies, affected the response rate. In patients with a KPS of 90%–100%, the response rate was 54% (13 responses among 24 patients), compared to 18% (three responses among 17 patients) ($P < 0.01$) in those with a KPS of $\leq$ 80%. No other prognostic indicator (eg, liver

function) affected response or survival. It should be noted that eight patients had objective CEA responses, five of whom had responses as defined by other criteria.

Toxicity was noteworthy but well-tolerated in most patients in this outpatient program. The mean leukocyte count nadir was 2800/mm^3 and the mean platelet count nadir was 86,000/mm^3. In most cases, myelosuppression was easily managed with dose modification. Eighteen percent of 49 patients had leukocyte count nadirs < 1900/mm^3 (one patient to < 1000/mm^3), and 22% had platelet count nadirs < 50,000/mm^3. Hemorrhage did not occur in any patient. Nonhematologic toxic effects included mild nausea (57%), moderate nausea and vomiting (22%), mucositis (30% mild, 8% severe), diarrhea (44% mild, 14% moderate), ataxia (4%), renal failure (8% moderate to severe) (*see* below), and esophagitis (2% mild, 2% severe).

Two toxic deaths were observed: one was an early death due to sepsis, hypotension, and respiratory distress; the other was a late death due to renal failure. One death was associated with drug-induced myelosuppression (leukocyte and platelet counts of 600 and 3000/mm^3, respectively). Infections developed in three patients.

Four patients developed abnormal renal function (creatinine > 2 mg/dl). One of these patients died of renal failure with concurrent thrombocytopenia and had received a total MMC dose of 100 mg. He was placed on hemodialysis but died from uremia. An autopsy revealed evidence of microangiopathic hemolytic uremia in the kidneys and no systemic malignancy.

DISCUSSION

The regimen used in this trial appears to be an active combination in advanced colorectal cancers and supports the proposed synergy between MTX and 5-FU. The addition of MMC to sequential MTX and 5-FU perhaps added to its overall activity, but since our study was nonrandomized, we are unable to conclusively document the role of this agent. Nevertheless, this three-drug combination resulted in a CR and PRs in nearly 40% of our patients, which is better than our previous experience with sequential MTX and 5-FU in which only 20% of the patients responded (9), and compares favorably with other reports (table 1).

One other study of this combination in patients with

TABLE 1.—Sequential MTX and 5-FU in colorectal cancer (long MTX pre-exposure)

	Active regimens (> 25% response)		
Investigator (ref No.)	Regimen*	MTX pre-exposure (hrs)	No. of responses/ No. of patients (%)†
Ignoffo et al (present study)	MTX, 30 mg/m^2 orally q 12 hrs × 2; MMC, 10 mg/m^2 by IVP; 5-FU, 500 mg/m^2 by IVP 12 hrs after MTX; LR, 20 mg/m^2 q 4 hrs × 4 starting 24 hrs after MTX	12	19/49 (39); MDR, 8.3 mos
Weinerman et al (18)	MTX, 20 mg/kg over 4 hrs q 3-4 wks; 5-FU, 600 mg/m^2 by IVP q 3-4 wks	4	10/29 (34); MDR, 13 mos
Herrman et al (15)	MTX, 200-300 mg/m^2 over 4 hrs q 2-3 wks; 5-FU, 900 mg/m^2 by IVP 3 hrs after MTX	7	5/14 (36)
Kemeny and Michaelson (21)	MTX, 40 mg/m^2 by IVP on Days 1 and 8; 5-FU, 600 mg/m^2 on Days 1 and 8, 24 hrs after MTX	24	8/24 (33)
Drapkin et al (16,22)	MTX, 200-600 mg/m^2 iv over 4 hrs q 2 wks; 5-FU, 300-600 mg/m^2 7 hrs after MTX q 2 wks	7	5/19 (26)
Moayeri et al (23)	MTX, 200 mg/m^2 over 4 hrs; 5-FU, 600 mg/m^2 over 3 hrs, 7 hrs after MTX	7	6/26 (33)
Mehrotra et al (24)	MTX, 100 mg/m^2 iv over 30 mins; 5-FU, 600 mg/m^2 by IVP at 4 hrs; LR, 25 mg/m^2 by IVP at 24 hrs, then 4 × day	4	8/10 (80)
Total:			61/171 (36)

*IVP = iv push; LR = leucovorin rescue.
†MDR = median duration of remission.

similar pretreatment characteristics has been recently reported by Biran et al (13). In 31 patients studied, no PRs and 18 minimal or stable disease responses were observed, with a median survival of 6 months (13). This regimen consisted of *concurrent* MTX and 5-FU plus leucovorin rescue and MMC in doses similar to those used in our study. Toxicity primarily consisted of severe myelosuppression resulting in 14 patients being hospitalized (ten for leukopenia and fever and four for bleeding) and one drug-related death in 135 courses of therapy. Thus, the sequential method of MTX and 5-FU administration as used in our study may be more effective in advanced colorectal cancer with less life-threatening toxicity compared to concurrent MTX and 5-FU and MMC combinations. However, a prospective comparative trial is necessary before a definitive conclusion can be made.

The reason for the higher response rate observed in this report compared to our previous study (9) and that of Biran et al (13) may be due to either the longer MTX exposure in the current regimen or the addition of MMC. This sequential method of MTX and 5-FU plus MMC and leucovorin led to a response rate of about 40% and is consistent with that of several other investigators using prolonged MTX pre-exposure. In contrast, with short MTX pre-exposure, objective responses appear to be no greater than with 5-FU alone or in combination with MMC (table 2). Recent studies by Burnet et al (25), Cantrell et al (17), and Weinerman et al (18) utilized a shorter MTX pretreatment of 1 and 4 hours, with variable response rates. Prospective controlled trials are necessary to determine if MTX pre-exposure periods of 24 hours are more effective than those of 4–7 hours (table 2). It is also uncertain if high doses of MTX as reported by Weinerman et al (18) are necessary. Thus, it is important that future studies with sequential MTX and 5-FU consider the duration of MTX pre-exposure as well as dosage.

Another factor which may affect fluoropyrimidine effectiveness in colorectal cancer is the concurrent use of folate. It has been shown in cell culture systems that the cytotoxic action of 5-FU is enhanced when reduced folates are present in high concentration (about 1×10^{-5} M) (6). Moreover, in human xenographs of colon carcinoma, binding of 5-FdUMP to thymidylate synthetase is maximal after the addition of exogenous folate (14). These factors were considered by Machover et al (14) in a study of 5-FU plus high-dose leucovorin. Their response

TABLE 2.—Sequential MTX and 5-FU in colorectal cancer (short MTX pre-exposure)

	Inactive regimens (< 25% response)		
Investigator (ref No.)	Regimen*	MTX pre-exposure (hrs)	No. of responses/ No. of patients (%)
Friedman et al (9)	MTX, 250 mg/m^2 over 3 hrs; 5-FU, 1 g/m^2 over 24 hrs	2	2/12 (17)
Biran et al (13)	MTX, 30 mg/m^2 on Days 1, 8, 29, and 36; 5-FU, 600 mg/m^2 by IVP on Days 1, 8, 29, and 36	Concurrent	0/31 (0)
Burnet et al (25)	MTX, 250 mg/m^2 by IVP q 3 wks; 5-FU, 60 mg/m^2 by IVP 1 hr after MTX q 3 wks	1	1/18 (5)
Cantrell et al (17)	MTX, 250 mg/m^2 by IVP q 2-3 wks; 5-FU, 600 mg/m^2 by IVP 1 hr after MTX q 2-3 wks	1	1/16 (6)
Blumenreich et al (26)	MTX, 200 mg/m^2 by IVP on Days 1 and 8; 5-FU, 600 mg/m^2 by IVP 1 hr after MTX on Days 1 and 8	1	0/9 (0)
Hansen et al (27)	MTX, 100-150 mg/m^2 iv over 60 mins on Days 1 and 8; 5-FU, 600 mg/m^2 iv over 60 mins on Days 1 and 8	1	5/20 (25)
Mahajan et al (28)	MTX, 800 mg/m^2 iv over 1 hr q 3 wks; 5-FU, 800 mg/m^2 iv over 1 hr q 3 wks	0 and 3	2/13 (16); 2/17 (12)
Rangineni et al (29)	MTX, 60 mg/m^2 by IVP; 5-FU, 1000 mg/m^2 by IVP q 3 wks	Concurrent	1/30 (3)
Total:			14/166 (8)

* IVP = iv push.

rate in metastatic colorectal carcinoma was 56% in 16 previously untreated patients, with a mean duration of response of 6+ months (14). In this study, the dose of leucovorin was 200 mg/m^2 given as a 15-minute infusion. In contrast, the dose and consequently the estimated serum concentration of leucovorin in this study would be about tenfold less. It is uncertain whether leucovorin administered in this study impacted on response or toxicity.

While overall toxicity was generally mild, it appears that MMC was responsible for the moderately severe myelosuppression, particularly thrombocytopenia, and the renal failure (including one fatality documented pathologically) observed in this study. The number and severity of toxic effects were unexpectedly high despite the use of ordinary doses of each agent in the regimen. With several previously reported cases of MMC-induced renal toxicity (19-21), we believe that this agent probably contributed to these cases of renal failure since no other causative factors were evident. The total dose of MMC in these four cases ranged from 35 to 100 mg (mean, 69), while the number of courses of treatment ranged from two to five.

CONCLUSIONS

This sequential MTX and 5-FU plus MMC and leucovorin regimen given on an outpatient basis is an effective combination in advanced cancer of the large bowel. Furthermore, it appears that at least 12 hours of pretreatment with oral MTX allows time to enhance biochemical synergy with 5-FU and appears to play a major role in the level of observed activity in our trial (response rate of 40%). Thus, sequential MTX and 5-FU plus MMC and leucovorin merits further study in advanced large bowel cancer. A variety of comparative studies could be explored to establish the optimal pre-exposure period for MTX, the benefit of added MMC, and the role of leucovorin in sequential MTX and 5-FU trials.

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Cytogenetic Effects of Amsacrine on Human Lymphocytes In Vivo and In Vitro[1]

Chien Song Kao-Shan,* Kenneth Micetich, Leonard A. Zwelling, and Jacqueline Whang-Peng[2,3]

Amsacrine (*m*-AMSA) is presently being utilized in phase I–II studies at the Medicine Branch, National Cancer Institute, National Institutes of Health (Bethesda, MD), and is being administered as a continuous infusion to patients with progressive malignancy after conventional therapy. In the present study, we examined the effects of this drug, in vivo and in vitro, on chromosomal morphology and the frequency of sister chromatid exchange (SCE) induction in human peripheral blood lymphocytes. In the in vivo studies, eight patients receiving 30 mg/m²/day of *m*-AMSA by continuous infusion showed increased levels of chromosomal aberrations, up to a maximum of 14% (median; range, 10%–24%) at 96 hours compared to 1% (median; range, 0%–4%) in the control group; no increase was noted in SCE frequencies. In vitro studies of normal human lymphocytes with various concentrations of *m*-AMSA, including doses comparable to those used in vivo, showed both increased levels of chromosomal aberrations, ranging from 8% to 100%, and increased SCEs, ranging from 1.5 times the normal at the lowest concentration studied (0.005 μg/ml) to 12 times the normal (0.25 μg/ml). In correlating SCEs with breaks in the in vitro studies, it was found that two-thirds of the total breaks occurred at SCE points; this indicates a close relationship between the events leading to these two types of aberrations. In vitro isotope incorporation studies showed inhibition of both RNA and DNA syntheses but no significant inhibition of protein synthesis; at a dose of 5.0 μg/ml, RNA synthesis was 80% inhibited and DNA synthesis was 60% inhibited after 36 hours of incubation with *m*-AMSA. The difference in the results between in vitro and in vivo SCE studies may be due to possible DNA repair in the in vivo-treated lymphocytes in *m*-AMSA-free medium in vitro, a process not available to the in vitro-treated cells. [Cancer Treat Rep 68:989–997, 1984]

Amsacrine (*m*-AMSA), an acridine derivative synthesized by Cain and Atwell (1), has proven to be an effective agent in the treatment of acute myelocytic leukemia (2–5). It binds strongly to DNA in vitro and intercalates between adjacent base pairs in a manner similar to the parent molecule, 9-aminoacridine (6). Recent studies of the effects of *m*-AMSA on cultured tumor cells indicate that prolongation of the drug treatment period substantially increased *m*-AMSA's cytotoxicity; this may be due to the short-lived effects of the drug on the tumor cells (7). In response to these findings, the Medicine Branch of the National Cancer Institute conducted a phase I–II study utilizing continuous infusion of *m*-AMSA in patients with progressive malignancy refractory to conventional therapy. There have been no cytogenetic reports in the literature concerning the in vivo effects of *m*-AMSA in humans. This study was conducted to study the damaging effects of *m*-AMSA on the genetic material of normal and neoplastic cells and to examine the significance of sister chromatid exchanges (SCEs) as indicators of DNA damage.

MATERIALS AND METHODS

Peripheral blood from seven normal donors, all of whom were nonsmokers with no known history of recent medication or chemical exposure, was used in the in vitro *m*-AMSA study. The peripheral blood from eight patients being treated with *m*-AMSA was examined to determine the in vivo effects of the drug; four of these patients had

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[2]Cytogenetic Oncology Section, Medicine Branch (*C. S. Kao-Shan* and *J. Whang-Peng*), and Laboratory of Molecular Pharmacology (*K. Micetich* and *L. A. Zwelling*), Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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Reprint requests to: Chien Song Kao-Shan, MD. Cytogenetic Oncology Section, Medicine Branch, National Cancer Institute, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, MD 20205.

lymphoma, three had carcinoma of the ovary, and one had rhabdomyosarcoma. Seven patients were studied during their first *m*-AMSA cycle, which consisted of a dose of 30 mg/m^2/day administered as a continuous iv infusion for 3 or 4 days. Two patients (Nos. 9 and 14) were also studied during subsequent *m*-AMSA cycles, and one patient (No. 13) was studied only during the third cycle. All of the patients had received other anticancer agents prior to treatment with *m*-AMSA. However, six of the eight patients had periods without any antitumor chemotherapy ranging from 6 weeks to 6 months before administration of *m*-AMSA. One patient (No. 14) had no antitumor therapy for 1 year and another patient (No. 15) had no antitumor therapy for 14 days. Radiation therapy was administered to two of the eight patients: Patient 13 received 3000 rads (pelvic) 2 months prior to *m*-AMSA and Patient 9 received 2400 rads (whole-brain with eye shield) 1 year prior to *m*-AMSA treatment.

In Vivo Cytogenetic and SCE Studies

Peripheral blood was collected with heparin (20 units/ml) from patients at 0, 24, 48, and 72, and/or 96 hours following the initiation of *m*-AMSA administration. The blood was allowed to stand for 30 minutes and the leukocyte-rich plasma was collected after gravity sedimentation of the erythrocytes. The blood leukocytes were washed twice with McCoy's 5A medium to avoid the presence of *m*-AMSA in the in vitro culture system. The cell count was adjusted to a final concentration of 1×10^6 wbcs/ml in McCoy's 5A medium containing glutamine, penicillin-streptomycin, 30% fetal bovine serum, and phytohemagglutinin (PHA) (0.1 ml of DIFCO PHA, cat #0528, reconstituted with 5 ml of distilled water, for each 10-ml culture), and was then incubated at 37°C for 3 days. 5-Bromodeoxyuridine (BrdUrd), at a final concentration of 10 μg/ml, was added 24 hours after culture initiation, and the incubation was continued for an additional 48 hours in the dark. All cultures were treated with demecolcine (0.1 μg/ml) for the final 2 hours of the incubation and harvested after a total incubation time of 72 hours; this procedure produces a substantial number of cells which have undergone two cycles of DNA replication. The resulting slides were air-dried, aged for from 1 week to 1 month, and stained with a conventional Giemsa stain or with Hoechst 33258 fluorescent stain plus Giemsa stain using the method of Goto et al (8).

In Vitro Cytogenetic, SCE, DNA, RNA, and Protein Synthesis Studies

Short-term leukocyte cultures from normal donors were set up to study the in vitro effects of *m*-AMSA on human lymphocytes. The concentrations of *m*-AMSA used were 0.005, 0.025, 0.05, 0.25, 0.5, and 1.0 μg/ml. These in vitro doses correlated with the in vivo drug levels, which were between 0.1 and 0.2 μM (approximately 0.04–0.08 μg/ml). The drug was added to the leukocyte cultures at 0 time (group I) or at 24 hours after culture initiation (group II). BrdUrd was added to the cultures for SCE studies as described above.

PHA-stimulated leukocyte cultures were established to study the effect of *m*-AMSA on protein, RNA, and DNA syntheses. Final in vitro concentrations of *m*-AMSA were 0.05, 0.5, and 5.0 μg/ml of culture; isotope (^{3}H-thymidine, ^{3}H-uridine, or ^{14}C-leucine) was added to each culture at a final concentration of 0.5 μCi/ml. *m*-AMSA and isotopes were added at 0, 12, 24, and 36 hours for 5-hour pulse treatments; duplicate samples were set up for each concentration and time point. The total radioactivity in the 10% trichloroacetic acid (TCA) precipitates was determined by liquid scintillation counting.

Conventional Cytogenetic Study

In both the in vivo and in vitro studies, the mitotic cells were examined for the presence of chromosomal abnormalities and aberrations; when possible, 50 metaphase chromosome spreads were analyzed from the conventional Giemsa-stained preparations of each sample. The aberrations were scored for frequency (%) of cells with chromosomal aberrations per sample. Any cell containing one or more of the following chromosomal aberrations was scored: chromatid break, chromosome break, chromosome translocation, chromatid exchange, ring formation, fragmentation, and pulverization.

Sister Chromatid Exchanges

Thirty metaphases in second division were scored for SCEs in each sample, if possible. SCE analysis was restricted to cells with 46 chromosomes. The number of SCEs per chromosome is quantified as the number of reciprocal exchange points along one of the sister chromatids. The mean of SCE frequencies per metaphase and the standard deviation were statistically calculated. In the in vivo study, Student's t test was employed to determine the significance of (*a*) the SCE frequencies of each patient at 0 hour compared to those of the normal control, and (*b*) the SCE frequencies at various time points after drug initiation (24, 48, 72, and 96 hours) for each patient compared to that patient's 0-hour sample. The t test was also applied to the in vitro study samples within each group.

RESULTS

In vivo effects.—The results of the in vivo chromosome aberration studies of the first treatment cycle and those of the normal control group are shown in table 1. The frequency of cells with chromosomal aberrations showed an increase at 24 hours (median, 10%; range, 4%–16%), and

TABLE 1.—Chromosomal aberrations in normal control group and in patients treated with *m*-AMSA

Control or patient No.	Treatment cycle	Sex/age (yrs)	Diagnosis	Frequency (%) of cells with chromosomal aberrations* Duration of infusion (hrs) 0	24	48	72	96
			Controls					
1		F/52	Normal	2	—	—	—	—
2		F/50	Normal	4	—	—	—	—
3		F/40	Normal	2	—	—	—	—
4		F/36	Normal	0	—	—	—	—
5		F/18	Normal	0	—	—	—	—
6		M/33	Normal	0	—	—	—	—
7		M/30	Normal	0	—	—	—	—
			Patients					
8	I	F/44	Lymphoma	4	4	6	12	—
9	I	M/52	Lymphoma	2	10	12	FM	14
	III			6	8	10	18	18
10	I	M/62	Lymphoma	6	16	14	FM	—
11	I	M/72	Lymphoma	4	10	28	NM	—
12	I	M/46	Rhabdomyosarcoma	NM	8†	14	6	10
13	III	F/65	Ovarian carcinoma	22	FM	26	NM	—
14	I	F/65	Ovarian carcinoma	2	4	6	16	—
	II			12	24	NM	26†	—
15	I	F/49	Ovarian carcinoma	6	12	16	18	24

* Including chromatid break, chromosome break, chromosome translocation, chromatid exchange, ring formation, fragmentation, or pulverization. — = not done; FM = few mitoses; and NM = no mitoses.

†No. of cells scored was < 50: Patient 11, 14 cells; Patient 12, 12 cells; Patient 14, 38 cells. 50 cells were scored for all other samples.

the highest frequency was observed at 96 hours (median, 14%; range, 10%-24%) compared to the normal control (median, 1%; range, 0%-4%) and 0-hour levels (median, 4%; range, 2%-6%). In summary, after one in vivo treatment cycle, *m*-AMSA causes increases in chromosomal aberrations, with the highest frequencies being observed at 96 hours. Three patients were studied during multiple cycles of *m*-AMSA (table 1). Two patients were studied during the first and second (Patient 14) or the first and third (Patient 9) cycles: in both patients, chromosomal aberrations were increased in the second cycles, including increased levels at 0 hour (12% and 6%, respectively). Patient 13 was studied only during the third *m*-AMSA cycle: total chromosomal aberrations were 22% at 0 hour and 26% at 48 hours.

In vitro effects.—The results of the in vitro chromosome studies with *m*-AMSA are shown in table 2; the drug was added at 0 hour in group I and at 24 hours in group II. In both groups, increasing the drug concentrations produced decreases in the mitotic indices (*group I*—from 36.4‰ in the control to 0‰ at 0.5 and 1.0 μg/ml; *group II*—from 47‰ in the control to 0.7‰ at 1.0 μg/ml). Increased chromosomal aberrations were also observed with increasing drug concentrations: in group I, 0%, 8%, and 56% of the cells had aberrations at drug concentrations of 0, 0.005, and 0.05 μg/ml, respectively; in group II, the aberrations ranged from 4% in the control to 100% at drug concentrations of 0.25, 0.5, and 1.0 μg/ml (fig 1, A, C, and E and fig 2).

Sister Chromatid Exchanges

In vivo effects.—The results of the SCE studies in the normal control group (seven individuals) and in the eight patients who received *m*-AMSA, including the three patients who received multiple cycles of the drug, are shown in table 3. Student's t-test determinations of SCEs showed no significant differences between seven of the patients prior to treatment and the normal control group ($P < 0.2$). There was also no significant difference between the initial sample and that obtained during treatment ($P < 0.1$). The only patient to show elevated SCE levels was No. 15, who had received other chemotherapy (CHEX-UP; cyclophosphamide, hexamethylmelamine,

TABLE 2.—Effects of *m*-AMSA on SCEs and chromosomal aberrations on human lymphocytes in vitro

Concentration* (μg/ml)	Time drug was added (hrs)	SCE/metaphase (mean ± SD)	Frequency (%) of cells with chromosomal aberrations†						Mitotic index (‰)
			ctb	csb	t/cte	r	f/p	tot	
			Group I						
0	0	7.60 ± 3.06	0	0	0	0	0	0	36.4
0.005	0	11.23 ± 3.06	4	2	0	0	2	8	30.0
0.05	0	38.56 ± 5.60†	26	6	12	0	12	56	10.6
0.5	0	No mitoses			No mitoses			—	0
1.0	0	No mitoses			No mitoses			—	0
			Group II						
0	24	9.27 ± 3.75	2	2	0	0	0	4	47.0
0.005	24	12.93 ± 4.32	6	4	0	0	0	10	50.0
0.025	24	22.83 ± 6.68	14	6	0	0	2	22	38.0
0.05	24	44.17 ± 8.71	18	8	6	0	16	48	30.0
0.25	24	112.4 ± 19.05†	12	0	86	0	2	100	6.3
0.5	24	Extensive fragmentation	0	0	0	0	100	100‡	2.0
1.0	24	Extensive fragmentation	0	0	0	0	100	100‡	0.7

*Final concentration of *m*-AMSA in culture.

†ctb = chromatid break; csb = chromosome break; t/cte = chromosome translocation or chromatid exchanges; r = ring formation; f/p = fragmentation or pulverization; tot = total chromosomal aberrations; — = not done.

‡No. of cells scored for SCEs was < 30: group I, 0.05 μg/ml, 25 cells; group II, 0.25 μg/ml, 10 cells. 30 cells were scored for all other samples. No. of cells scored for chromosomal aberrations was < 50: group II, 0.5 μg/ml, 7 cells; 1.0 μg/ml, 2 cells. 50 cells were scored for all other samples.

cisplatin, and 5-FU) 14 days prior to *m*-AMSA treatment. Both her pretreatment and during-treatment samples were elevated: the pretreatment sample was significantly elevated (mean, 17.64 SCEs/metaphase) above the control levels ($P < 0.001$), and the during-treatment samples were significantly increased (25.10–28.71 after 24, 48, 72, and 96 hours) over her pretreatment samples ($P < 0.005$).

In vitro effects.—SCE levels were shown to increase with increasing in vitro *m*-AMSA concentration (table 2; fig 1, B, D, and F). Student's t-test comparisons showed that the increases were significant in both groups I and II ($P < 0.001$). In both groups, SCEs could not be enumerated at the concentrations of 0.5 and 1.0 μg/ml because there were no mitoses or the chromosomes were extensively fragmented and the mitotic indices were low. Detailed analyses of chromatid or chromosome breaks and SCEs in the same cells were performed on a total of 22 metaphases in one of the samples treated with 0.25 μg/ml of *m*-AMSA. A total of 121 breaks and 2233 SCEs were observed in these 22 metaphases; 78 of the breaks were located at SCE points, indicating that of all the breaks in these cells, 64.5% were concomitant with SCEs.

Isotope Incorporation Study

The in vitro effects of *m*-AMSA on DNA, RNA, and protein syntheses using ^{3}H-thymidine, ^{3}H-uridine, and ^{14}C-leucine incorporation (5-hour pulse labels) were studied. No significant effects on protein synthesis were noted after 0, 12, and 24 hours of culture with *m*-AMSA at final concentrations of 0.05, 0.5, and 5.0 μg/ml; inhibitory effects (up to 50%) were, however, seen at 36 hours with a concentration of 5.0 μg/ml. Inhibition of RNA synthesis was noted at all concentrations at all time points: at 36 hours, there was 55% inhibition at a concentration of 0.5 μg/ml and 80% inhibition at a concentration of 5.0 μg/ml. Inhibition of DNA synthesis was also noted, beginning at 24 hours with the highest concentration (30% inhibition at 5.0 μg/ml); at 36 hours, inhibition was noted at both 0.5 μg/ml (30%) and 5.0 μg/ml (60%). No inhibition of DNA synthesis was noted at any time with the lower concentrations.

DISCUSSION

For a variety of cytogenetic and isotope incorporation studies, it is difficult to obtain the neoplastic cells, whether of leukemic or solid tumor origin, which are the target of *m*-AMSA chemotherapy regimens. Instead, the cell selected for these studies was the lymphocyte, which is readily available and which is sensitive to the clastogenic effects of a variety of drugs, as well as to their disruptive effects on the cell cycle.

m-AMSA is known to preferentially bind to cellular DNA (9). Deaven et al (10) studied the effects of *m*-AMSA on Chinese hamster cells and found cell-cycle-specific chromosome damage; the drug was particularly effective

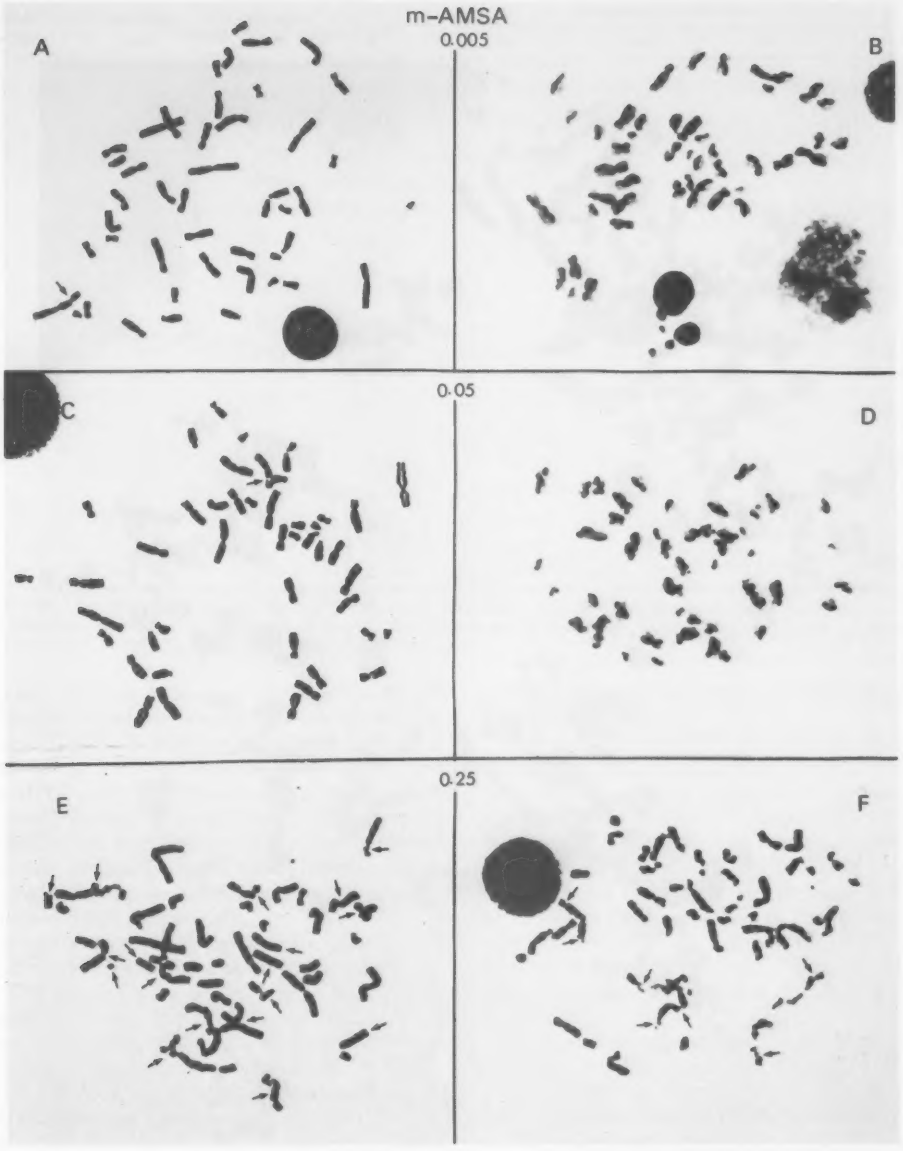

FIGURE 1.—Chromosomal aberrations and SCEs in in vitro group II following treatment with *m*-AMSA at concentrations of 0.005, 0.05, and 0.25 μg/ml. A,C,E: chromosomal aberrations are indicated by arrows; conventional Giemsa stain. **A.** Chromatid break; *m*-AMSA concentration of 0.005 μg/ml. **C.** Triradial configuration, classified as chromatid exchange; *m*-AMSA concentration of 0.5 μg/ml. **E.** Cell with chromatid breaks, chromosome breaks, chromosome translocations, chromatid exchanges, and fragmentation; *m*-AMSA concentration of 0.25 μg/ml. B,D,F: SCEs; Hoechst 33258 fluorescent stain plus Giemsa stain. **B.** Metaphase with 20 SCEs; *m*-AMSA concentration of 0.005 μg/ml. **D.** Metaphase with 49 SCEs; *m*-AMSA concentration of 0.05 μg/ml. **F.** Metaphase with 102 SCEs; *m*-AMSA concentration of 0.25 μg/ml; breaks located at SCE points are indicated by arrows.

FIGURE 2.—Chromosomal aberrations (extensive fragmentation) in in vitro group II treated with *m*-AMSA concentration of 0.5 μg/ml; conventional Giemsa stain.

TABLE 3.—SCEs in normal control group and patients treated with *m*-AMSA

Control or patient No.	Treatment cycle	Sex/age (yrs)	SCE/metaphase (mean ± SD)* Duration of infusion (hrs) 0	24	48	72	96
			Controls				
1		F/52	6.81 ± 2.44	—	—	—	—
2		F/50	9.27 ± 3.75	—	—	—	—
3		F/40	7.94 ± 3.76	—	—	—	—
4		F/36	8.83 ± 3.46	—	—	—	—
5		F/18	7.60 ± 3.05	—	—	—	—
6		M/33	8.22 ± 2.45	—	—	—	—
7		M/30	7.08 ± 2.51	—	—	—	—
			Patients				
8	I	F/44	9.77 ± 4.32	10.63 ± 3.92	10.66 ± 4.80	9.67 ± 4.50	—
9	I	M/52	10.77 ± 4.39	10.23 ± 4.36	8.97 ± 3.51	FM	8.94 ± 2.69†
	III		10.07 ± 6.90	13.77 ± 9.10	9.37 ± 7.29	12.37 ± 3.92	10.81 ± 4.71
10	I	M/62	9.42 ± 3.17	10.77 ± 3.94	9.32 ± 3.12	FM	—
11	I	M/72	10.73 ± 5.02†	15.72 ± 9.70†	FM	NM	—
12	I	M/46	NM	FM	8.27 ± 3.33	8.80 ± 3.08	10.91 ± 3.88†
13	III	F/65	9.31 ± 3.91†	FM	9.97 ± 5.24	NM	—
14	I	F/58	10.33 ± 6.71	11.13 ± 5.86	12.71 ± 6.07	12.38 ± 5.68	—
	II		7.75 ± 2.25†	9.83 ± 3.80	NM	No 2nd division	—
15	I	F/49	17.64 ± 7.61†	27.35 ± 9.81	28.57 ± 10.24	25.1 ± 9.61	28.71 ± 9.63

*— = not done; FM = few mitoses; NM = no mitoses; No 2nd division. None of the cells were in 2nd division.

†No. of cells scored was < 30: Patient 9, 16 cells; Patient 11, 0 hr, 15 cells and 24 hrs, 10 cells; Patient 12, 22 cells; Patient 13, 26 cells; Patient 14, 8 cells; Patient 15, 14 cells; 30 cells were scored for all other samples.

when chromatin was undergoing structural modifications (during G_1-S and S-G_2 phases). Furlong et al (11) observed that L1210 cells were ten times more sensitive to *m*-AMSA during the S-G_2 period in the cell cycle, and Tobey et al (12) noted that, in its effect on cycling cells, it closely resembles the drug doxorubicin. Our results in the short-pulse isotope labeling experiments clearly showed that *m*-AMSA inhibits DNA and RNA syntheses, a dose of 0.5 μg/ml reducing the rate of synthesis to approximately 30% and 55%, respectively.

The in vivo clastogenic properties of *m*-AMSA were studied in eight patients with a variety of neoplastic disorders. The samples were obtained prior to *m*-AMSA treatment and during the first, second, and/or third treatment cycles (none of the patients studied achieved either partial or complete remissions following *m*-AMSA treatment). The drug was shown to cause a significant increase in chromosomal aberrations. One patient, No. 13, who had a high base level (22%) of aberrations, showed an even higher level (26%) in samples obtained during the third infusion cycle. The increased initial aberration level seen in this patient could very well be due to the radiation therapy which she received 2 months prior to *m*-AMSA administration, since radiation is known to produce chromosomal aberrations (13,14), or it could be due to the fact that she received two cycles of *m*-AMSA. The additive effects of *m*-AMSA and irradiation were not tested in this study.

In vivo and *in vitro* SCE studies were conducted with lymphocytes from both normal individuals and patients in an attempt to assay the degree of chromosomal damage produced by *m*-AMSA. Patient 15 had a high level of SCEs (mean, 17.64/metaphase) prior to treatment which was increased to an even higher level of 28.71/metaphase after 96 hours of infusion. The increased initial level of SCEs seen in this patient may be due to treatment with other chemotherapeutic agents (CHEX-UP) received 14 days prior to *m*-AMSA infusion. The remaining seven patients who received *m*-AMSA in vivo showed no increase in SCEs after in vitro drug-free culture.

The type of damage produced or the recovery pathway employed in DNA repair may depend on the drug to which the cells are exposed. Raposa (15) studied the effects of cytostatic drugs on SCEs by examining the levels in PHA-stimulated lymphocytes (cultured in vitro) obtained from patients treated with a variety of such agents. He observed that a combination of cytarabine and 6-thioguanine did not induce higher frequencies of SCEs, whereas cyclophosphamide, given alone or in combination with other cytostatic drugs, produced a higher incidence of SCEs during the period of drug administration, with a return to the initial (pretreatment) levels approxi-

mately 10 days following cessation of treatment. The patient (No. 15) in the present study received CHEX-UP treatment terminating 14 days prior to *m*-AMSA administration: her pretreatment *m*-AMSA sample showed elevated SCEs, which were further elevated during and after *m*-AMSA treatment. This finding indicates an additive effect on SCEs from the two treatment combinations.

The results of our in vitro studies of normal lymphocytes, which showed a significant increase in chromosomal aberrations and SCEs, confirmed the findings of Crossen (16) who also observed an increase in SCEs after in vitro exposure to *m*-AMSA. Our results with PHA-stimulated lymphocytes, which has been exposed to the drug in vivo, showed an increase in chromosomal aberrations but no increase in SCEs. There are several possible explanations for the differences seen in vitro and in vivo. Raposa (15) postulated that they can be attributed to the fact that the various lymphocyte subpopulations apparently show different sensitivities to mutagens, some populations not even dividing in the presence of the cytostatic agents. They could also be due to the type of DNA effect produced by *m*-AMSA, a drug which binds strongly to DNA and intercalates between adjacent base pairs (10). By using the filter elution method, Zwelling et al (7) showed that *m*-AMSA produced single-strand and double-strand breaks in DNA in mouse leukemia L1210 cells. This could explain our results with *m*-AMSA treatment in vitro, where increased levels of both SCEs and aberrations were observed. The in vivo-exposed lymphocytes were stimulated (by PHA) to divide in drug-free medium, possibly allowing the reversal of some of the *m*-AMSA effects (7) during the replication cycle. This would explain the increased breakage and fragments with no elevation of SCEs in the in vivo-exposed lymphocytes. However, this postulation remains to be proved.

The correlation of SCEs and breaks was examined in our in vitro *m*-AMSA-treated samples. Random metaphases from the 0.25-μg/ml sample were selected and examined for both breaks (chromatid and chromosome) and SCEs: 64.5% of the breaks were located at SCE points, representing 3.5% of the total SCEs scored in these cells. There were obviously many more SCEs than breaks in these *m*-AMSA-treated cells. Galloway and Wolff (17) studied the relationship between chemically induced SCEs and chromatid breaks in both Chinese hamster ovary cell and rat gliosarcoma lines; the chemical agents used were doxorubicin, mitomycin, ethyl methanesulphonate, cyclophosphamide, bleomycin, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and DMBA. They found that 25% of the 914 breaks scored were associated with SCEs. The proportion of breaks accompanied by SCEs was related to the overall SCE frequencies and falls within the range predicted, based on the assumption that breaks and SCEs occur independently. Wolff (18) also stated that aberrations are most likely the result of double-strand breaks in DNA, whereas SCEs come from unremoved adducts. The fact that two-thirds of the total breaks in our in vitro *m*-AMSA-treated sample were noted in SCE regions indicates that there is some sort of close relationship between the events leading to these two types of aberrations.

From this study, we conclude that *m*-AMSA is a potent inhibitor of DNA and RNA, one which also produces damage to DNA. At low doses, it causes breaks which are reversible in drug-free environment, and at high doses or with long-term continuous low doses, it causes breaks which are not readily reversible. Our data showed a significant correlation between elevated SCEs and increasing concentrations of *m*-AMSA in vitro. The production of SCEs is a significant event and occurs at lower concentrations than do other aberrations, such as breaks. It is therefore a valuable test in the study of damage to genetic material.

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Amelioration of Cisplatin-Induced Nephrotoxicity by the Diuretic Acetazolamide in F344 Rats[1]

Nahed M. Osman, Marion P. Copley, and Charles L. Litterst[2,*]

Renal toxicity following a single iv nonlethal dose (6 mg/kg) of cisplatin (CDDP) was investigated in male F344 rats pretreated with 20 mg/kg of acetazolamide (ACZ) sc 30 minutes before CDDP administration. There was a decrease in nephrotoxicity as indicated by a smaller elevation of BUN, a milder histopathologic lesion, and a more rapid recovery of BUN in ACZ-pretreated animals. Renal platinum content of rats receiving ACZ was significantly lower than that in animals treated with CDDP alone. There was no change in platinum level in the plasma or the liver but there was a decreased platinum excretion in urine during 24 hours after treatment. Reduction of platinum concentration in the kidney might account for protection against the renal toxicity. The diuretic ACZ might be useful in the hydration regimens used clinically to prevent renal toxicity from CDDP. [Cancer Treat Rep 68:999–1004, 1984]

Cisplatin (CDDP) is an important anticancer drug that has exhibited activity against a wide variety of human malignancies (1). It is excreted by the kidney via a complex process that includes filtration, secretion, and reabsorption by the organic acid transport system (2,3). Despite significant antineoplastic activity of this drug, its clinical use is limited due to its dose-related nephrotoxicity (4,5).

Clinical interest in CDDP increased considerably after the publication of several reports indicating that induction of diuresis prior to CDDP administration ameliorated the renal damage caused by the drug (6–8). Cvitkovic et al (9) found that prehydration or mannitol administration with CDDP prevented the rise in BUN and serum creatinine which occur with CDDP administration in dogs. Ward et al (10) showed that furosemide protects against nephrotoxicity induced by CDDP in F344 rats. However, Pera et al (11) reported a progression of renal injury in furosemide-treated rats compared to those receiving CDDP alone. Their data suggest that furosemide should be contraindicated for use in prevention of CDDP-induced nephrotoxicity. Another study has also shown that mannitol and furosemide aggravate rather than ameliorate nephrotoxicity produced by toxic doses of CDDP (12).

The carbonic anhydrase inhibitor acetazolamide (ACZ) offers several potential advantages to recommend it as a diuretic for use with CDDP. First, it is an organic acid and would be expected to compete with CDDP for reabsorption, thus potentially decreasing the amount of dose reabsorbed and promoting excretion. Second, it produces an alkaline urine by virtue of its carbonic anhydrase-mediated increase in bicarbonate excretion. This is advantageous because it has been shown that CDDP excretion is highly favored by alkaline pH (13). Finally, the drug has two sulfur atoms in its structure which might be possible sites for chemical interaction with CDDP. The present study was therefore undertaken to investigate the effect of ACZ on the nephrotoxicity and disposition of CDDP in rats.

MATERIALS AND METHODS

Male Fisher 344 rats (Taconic Farms, Germantown, NY), weighing from 175 to 200 g, were housed individually in stainless-steel metabolism cages to allow separate collection of urine and feces. A 3–4-day acclimation period was allowed before initiation of the experiment. Animals had continued free access to Purina rat chow and tap water.

CDDP was obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD,

[1]Received Apr 29, 1983; revised Feb 16, 1984; accepted Mar 19, 1984.

[2]Laboratory of Medicinal Chemistry and Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

Reprint requests to: Charles Litterst, PhD, Laboratory of Medicinal Chemistry and Pharmacology, National Cancer Institute, Bldg 37, Rm 6D28, National Institutes of Health, Bethesda, MD 20205.

and was prepared immediately before injections at a concentration of 1 mg/ml in 0.9% NaCl solution. The diuretic effect of ACZ (Diamox; Lederle Laboratories, Pearl River, NY) was determined by treating rats with doses ranging from 5 to 25 mg/kg and by monitoring urine flow for 24 hours afterward. Maximum diuretic effect was obtained with 20 mg/kg and because this dose was consistent with other reports of its use in animals (14), it was selected for use in all subsequent studies. Perfix fixative was obtained from Fisher Scientific Co, Pittsburgh, PA.

Animals were divided into four groups. The first group (control) was injected with saline in a volume equal to that received by the drug-treated groups. The second group of animals (CDDP) was injected iv in the tail vein with 6 mg/kg of CDDP. The third group (ACZ) was injected sc with 20 mg/kg of ACZ. The fourth group (ACZ + CDDP) was given 20 mg/kg of ACZ sc 30 minutes prior to administration of 6 mg/kg of CDDP iv. All animals were lightly anesthetized with ether during the injections. Five animals from each group were kept in plastic cages and killed 4 days after treatment. Blood was collected in a heparinized syringe from the inferior vena cava and plasma was immediately separated. Liver and kidneys were removed and weighed. One kidney was fixed in Perfix solution and sectioned at 5 μm, and stained sections (hematoxylin and eosin) were subsequently prepared for histologic evaluation. A small portion of each tissue was digested in 10 volumes of nitric acid for evaluation of tissue platinum concentration.

Five animals from each group were kept individually in metabolic cages. Urine was collected at 30, 90, and 150 minutes; 24 hours; and then daily for 7 days. On Day 7, animals were killed by ether overdose, and blood and tissues were collected and analyzed as described. Plasma was used for platinum analysis and for determination of BUN using the diacetyl monoxime method as described by Crocker (15). Urine volume was measured daily. Platinum concentration in plasma and tissue was estimated by atomic absorption spectrophotometry (16). The results were statistically evaluated using Dunnett's t test at $P \leq 0.05$ (17). All data are $\bar{X}$ ± SD (n = 5) except for all 7-day CDDP and ACZ plus CDDP parameters, where n = 4.

RESULTS

BUN values obtained from animals treated only with ACZ (15.7 ± 2.3 mg/dl) were not statistically different from the controls (18.0 ± 1.6 mg/dl). Rats treated with CDDP had BUN values of 102.0 ± 19.5 and 109.3 ± 39.0 mg/dl on Days 4 and 7, respectively. Those values were significantly higher than those for the control (17.9 ± 1.7 mg/dl). On Day 4, BUN values from the ACZ plus CDDP group were less (70.4 ± 29.4) than those from the CDDP group, but were still significantly higher than the control. However, on Day 7 BUN in the ACZ plus CDDP group dropped to 20.1 ± 2.7 mg/dl. This value was not significantly different from the control, but was significantly less than that in the CDDP group (109.3 ± 39.0 mg/dl). It should be noted that at both 4 and 7 days after treatment the BUN values from ACZ plus CDDP-treated animals were significantly less than those from CDDP-treated animals. At 7 days the BUN values from ACZ plus CDDP-treated animals had returned to control levels.

Table 1 shows the platinum concentrations in plasma, liver, and kidney. Data show that plasma and liver concentrations of platinum were similar in animals given CDDP with or without ACZ. However, the total renal concentration was significantly higher in CDDP animals than in ACZ plus CDDP-pretreated animals on Day 4 but not on Day 7.

Figure 1 shows urine volume in the different treatment groups. In CDDP-treated animals urine volume increased on Day 2, returned to baseline on Day 4, and increased by more than eight times the control value by Day 7. In ACZ-pretreated animals there was less increase in urine volume on Days 1 and 7 compared to CDDP-treated animals.

Figure 2 shows the cumulative percent of the administered dose which was excreted in the urine. The pattern of elimination of platinum was biphasic in both treatment groups, with the highest amount being excreted during the first 24 hours. Although not shown

TABLE 1.—Platinum concentration in plasma and tissue from rats receiving CDDP alone or with ACZ*

Treatment	Day of platinum analyses†	Platinum (μg/ml)	Platinum (μg/g of wet tissue weight)	
		Plasma	Liver	Kidney
CDDP	4	0.36 ± 0.05	2.40 ± 0.92	13.20 ± 0.60
ACZ + CDDP	4	0.25 ± 0.17	2.50 ± 0.73	8.70 ± 2.70‡
CDDP	7	0.12 ± 0.00 (4)	2.70 ± 0.51	6.17 ± 0.83
ACZ + CDDP	7	0.10 ± 0.04 (4)	1.70 ± 0.61	7.00 ± 1.48

*Each value is the mean ± SD of 5 rats unless otherwise indicated in parentheses.
†No. of days from treatment until rats were killed.
‡Significantly different from CDDP on Day 4 at $P < 0.05$.

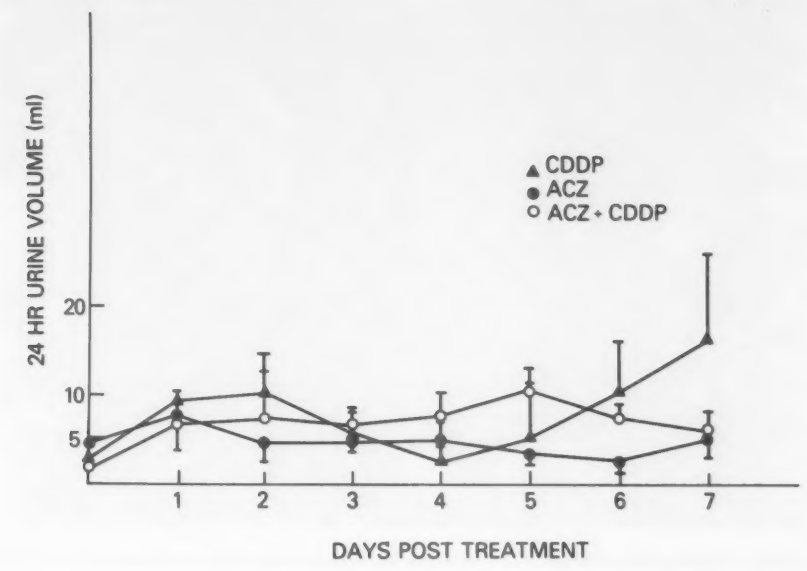

FIGURE 1.—Effect of pretreatment with ACZ (20 mg/kg sc) on 24-hr urine volumes in rats receiving CDDP (6 mg/kg iv). Values = mean ± SD.

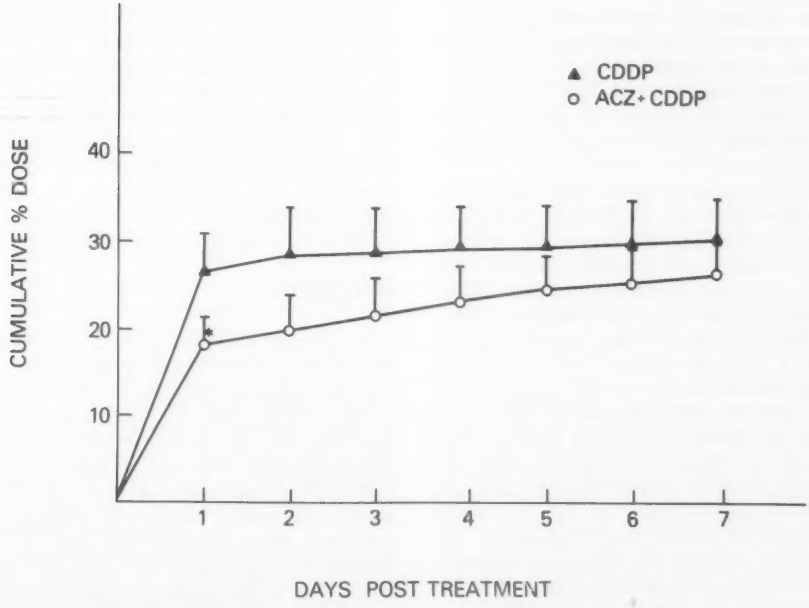

FIGURE 2.—Cumulative urinary excretion of platinum in rats treated with CDDP (6 mg/kg iv) alone or with ACZ (20 mg/kg sc). Values = mean ± SD. * = significantly different from CDDP at $P < 0.05$.

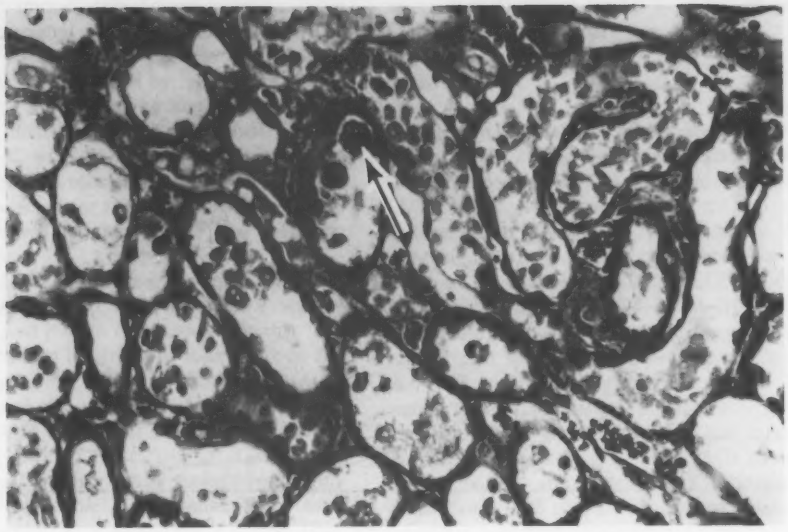

FIGURE 3.—Histopathologic changes at the corticomedullary junction in rats 7 days after treatment with CDDP (6 mg/kg iv) include pyknosis, karyorrhexis, karyolysis, cytoplasmic eosinophilia, sloughed cells in tubular lumina, flattened or no tubular epithelium, tubular dilation, and giant cells (arrow), probably in mitosis (magnification, × 280).

on the graph, platinum excretion was greatest during the first 30 minutes after treatment. After Day 3 there was a noticeable increase (twofold to threefold) in platinum excretion in the ACZ plus CDDP group relative to the excretion rate of the CDDP alone group, which remained essentially constant after the first day. The total amount of platinum excreted in urine in both groups during the 7-day period, however, was not significantly different.

In CDDP-treated animals on Day 4 there was a 20% loss of body weight. There was a 26% total weight loss by Day 7. On Day 4 the ACZ-pretreated animals showed a similar weight loss. However, on Day 7 the ACZ-pretreated group had regained weight, for a total loss of only 3%. One rat from each treatment group died on Day 4 after treatment. Both animals had severe oliguria for 1 day prior to death.

Four days after treatment with CDDP alone, a moderately severe acute tubular nephrosis (severity index = +4) appeared at the corticomedullary junction. This was characterized by cellular swelling and degeneration, karyorrhexis, aberrant mitotic figures, multifocal proximal tubular necrosis, tubular dilation, and slight protein leakage into the tubular lumen. Lesions on Day 4 in ACZ plus CDDP-treated rats were only moderate (severity index = +3) and were characterized by cellular swelling, degeneration, multifocal proximal tubular necrosis, and minimal tubular dilation. By Day 7 (fig 3), the corticomedullary junction nephrosis in CDDP-treated animals had become severe (severity index = +5), with hydropic degeneration, some interstitial hemorrhage, protein casts, tubular dilation, flattened tubular epithelium, necrotic cells and debris in the lumina, aberrant mitotic figures, and multifocal tubular necrosis extending into the renal cortex. In the ACZ-pretreated group (fig 4), however, the lesion was still only moderate (severity index = +3) and resembled that of Day 4 CDDP-treated animals with little karyorrhexis and with no protein casts. The ACZ-pretreated kidney appeared to be either less seriously affected by CDDP or undergoing regenerative changes by Day 7.

DISCUSSION

The present work showed that ACZ decreased renal damage induced by CDDP as indicated by less severe and less prolonged increase in BUN, a less severe or possibly regenerating histopathological lesion, and by an earlier recovery of body weight. In addition, kidney platinum content was less on Day 4 in the ACZ plus CDDP group and tended to correlate with the histopathological results. Kidneys of rats given CDDP alone contained more platinum and exhibited more renal damage than did kidneys of ACZ plus CDDP-treated rats at Day 4. The presence of less platinum in the kidney of ACZ-treated animals was a distinct advantage over data reported with the coadministration of other diuretics (11). In mannitol-treated rats, the platinum

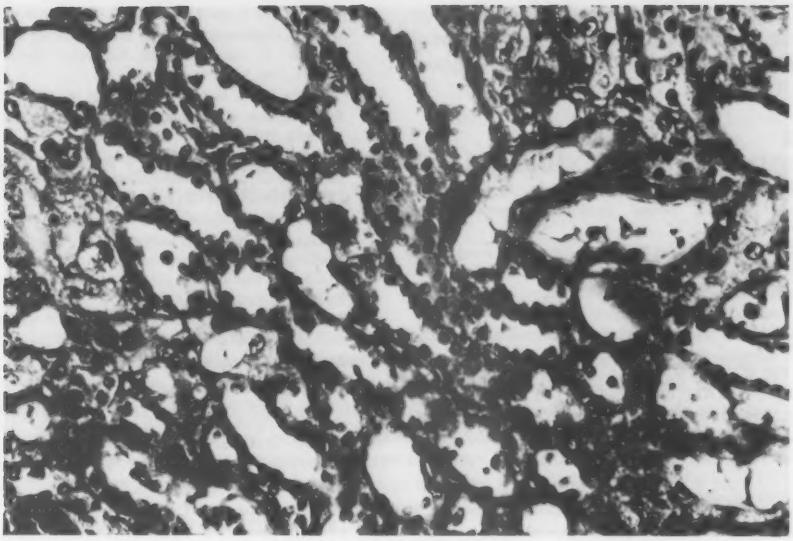

FIGURE 4.—Histopathologic changes at the corticomedullary junction 7 days after treatment with CDDP (6 mg/kg iv) + ACZ (20 mg/kg sc) show less tubular dilation, less necrosis of epithelial cells, large nuclei, more prominent nucleoli, and basophilic cytoplasm (magnification, × 280).

content of the kidney was shown equal to that of animals treated with CDDP alone, and a higher concentration of platinum was reported in kidneys of furosemide-pretreated animals that exhibited more serious renal lesion than did the CDDP only group (11). Because severity of renal lesions has previously been shown to correlate with renal platinum concentration (18), the decreased severity of the renal histologic lesion at Day 4 in ACZ plus CDDP-treated animals could reasonably be attributed to the decreased renal platinum concentration.

Protection of the kidney from CDDP-induced toxicity by ACZ was accompanied by a decrease in urinary platinum excretion in the first 24 hours, which was different from data observed in dogs with mannitol (9) and in rats with mannitol and furosemide (11). The reason for this decrease in platinum excretion in the face of increased urine volume is unexplained, particularly because dilution of urinary platinum in the kidney has been assumed to be the mechanism of diuretic protection against CDDP nephrotoxicity (19). However, ACZ has been reported to produce a mild decrease in glomerular filtration rate (18,20) and the decreased excretion might be related to the decreased mass flow of platinum through the glomeruli.

ACZ is a moderate diuretic and manifests its diuretic effect through inhibition of renal carbonic anhydrase with excretion of sodium bicarbonate (21). It has been shown that alkaline urine facilitates platinum excretion (13), even though this was not observed in this study. Because we did not measure urinary pH, it is possible that the single dose of ACZ was insufficient to alter the urinary pH enough to promote platinum excretion.

The biphasic polyuria (22) usually associated with CDDP treatment was less obvious than expected in the ACZ-treated animals, even with the diuretic effect of ACZ. The diuretic action of ACZ therefore may not be the sole mechanism modifying CDDP nephrotoxicity. ACZ is an organic acid and is transported by the renal tubules via the organic acid transport system (23). Another organic acid, probenecid, when given 1 hour prior to CDDP, appreciably reduced nephrotoxicity, presumably by its reported ability to decrease tubular reabsorption of platinum (24). It may be that ACZ acts similarly.

The concentration of platinum in plasma and liver was not altered by diuretic administration, even at Day 4. Thus, it seems unlikely that ACZ diuresis alters the disposition of platinum outside the kidney.

Various sulfur-containing compounds have been found to be effective in blocking or reversing the toxicity of CDDP (25,26) and a methionine-platinum complex has been determined to be an excretory product following CDDP administration (27). It is therefore possible that by virtue of the sulfur atoms in the ACZ structure, ACZ may also act to reduce toxicity by chemical interaction with the nucleophilic sites on the CDDP molecule.

The present study has shown that ACZ administration 30 minutes before CDDP significantly protects ani-

mals against nephrotoxicity observed when the drug was given alone and may be considered as a potential diuretic for clinical use in hydration-induced support for CDDP treatment.

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Examination of the Correlation of First-Week Mortality With the Gastrointestinal Syndrome Following Chemotherapy[1,2]

J. V. Moore[3,4,*]

The equation frequently made between mortality of mammals within the first week after treatment by cytotoxic drugs and the "gastrointestinal syndrome" has been critically examined. Six clinically used drugs were employed. Only for carmustine and 5-FU was a plateau in survival time observed, for increasing single doses. With cyclophosphamide or dactinomycin, mean survival time in the period of interest decreased sharply with dose, in marked contrast to the constancy observed over a large range of doses of radiation. The dynamics of expression of intestinal injury, as judged by mitotic activity in the crypts, was the same for all drugs and for radiation. However, the clonogenic response of the intestine (crypt microcolony assay) differed widely between agents. Cyclophosphamide and dactinomycin did not destroy any crypts at dose ranges containing the 50% lethality dose for mice. Of the remaining four drugs (carmustine, 5-FU, mechlorethamine hydrochloride, and doxorubicin), the surviving fraction of crypts corresponding to 50% lethality of mice varied 17-fold. Altering the route of administration of mechlorethamine hydrochloride from ip to iv increased the lethality dose and the dose for a given level of crypt survival, but *not* by the same factor. Survival of primitive, clonogenic cells of the bone marrow was also altered, but in the *opposite* direction. It is concluded that even where mean survival times are closely similar to radiation, the interpretation of the gastrointestinal syndrome following radiation cannot be applied quantitatively in a similar manner after many cytotoxic drugs. [Cancer Treat Rep 68:1005–1013, 1984]

In the literature of experimental chemotherapy, it is frequently said or assumed that animals dying within the first week after treatment are undergoing "gastrointestinal (GI) death" (1-4). In many cases, the basis for assuming death to be the result of a GI syndrome is that the mean survival time (MST) is the same as that after high doses of radiation. The gross and cellular phenomena associated with the radiation-induced GI syndrome have been studied extensively and are briefly summarized below.

(*a*) Over the approximate range of 10–100 Gy of low-LET radiation delivered to the whole body, the survival time of rodents is "constant," ie, reaching a plateau somewhere between 3 and 8 days, depending on species and strain (fig 1A; refs 5,6).

(*b*) At these radiation doses, there is a rapid reduction in proliferative activity (fig 1C) and onset of histological cell death in the epithelium of the crypts of the small intestine. Death of the animal is associated with denudation of the intestinal mucosa as the radiation-resistant maturing cells in the crypt and mature cells on the villus complete the transit of the villus within their normal time scale (1–2 days) but are not replaced (6). Death occurs soon after from dehydration, electrolyte imbalances, and bacterial attack.

(*c*) Doses > 7–8 Gy of low-LET radiation (or 3–4 Gy of neutrons) cause the complete ablation of some crypts within 2 days, while surviving crypts regenerate from one or more (clonogenic) cells, such that by 3 or 4 days, crypt cellularity may be three times that in untreated animals (figs 1C, 1D; refs 7–9).

(*d*) The ratio of doses of low- and high-LET radiations that result in a 50% surviving fraction of crypts at 3–4 days appears to correspond closely with the ratio of doses

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[3]Paterson Laboratories, Christie Hospital and Holt Radium Institute, Withington, Manchester, England.
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[*]*Reprint requests to:* Dr. J. V. Moore, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Withington, Manchester M20 9BX, England.

that cause GI death in 50% of a group of animals (the LD_{50} doses) within 1 week of irradiation (figs 1B, 1D; ref 10). Broadly, the survival of > 35%-50% of crypts at 3-4 days permits the majority of animals to survive the GI syndrome; less than this survival of crypts results in GI death (10-12).

This paper examines some of the gross and cellular phenomena associated with early death of mice following treatment by cytotoxic drugs that are used clinically, and discusses the limitations of the parallels that are often drawn between this mode of death and that described above following irradiation.

MATERIALS AND METHODS

Mice

Male, first-generation hybrid mice of the C57BL/6 × DBA/2 cross ($B6D2F_1$) were obtained from the Paterson Laboratories breeding colony. Experimental animals were used at 9-11 weeks of age, when mean weight was 25 g (2 SD = 3 g). The mice were kept under a 12-hour dark (6 PM to 6 AM), 12-hour light regimen, housed in boxes of six, and were allowed a standard mouse diet (Labsure, Poole, England) and tap water ad libitum.

Drugs

Unless otherwise indicated, all drugs were dissolved and diluted in sterile, 0.9% saline. Drugs used were: carmustine (BCNU; Bristol Labs, Syracuse, NY) dissolved in absolute ethanol and diluted in saline; mechlorethamine hydrochloride (HN_2; Boots, Nottingham, England); cyclophosphamide (CY; Ward Blenkinsop, Wembley, England); doxorubicin (ADR; Montedison, Barnet, England); 5-FU (Roche, Welwyn Garden City, England); and dactinomycin (ACT D; Merck and Co, Rahway, NJ). Drug concentrations (milligram of agent per kilogram of individual mouse body weight) were administered in injection volumes of 0.4-0.5 ml. Injections were always given ip at

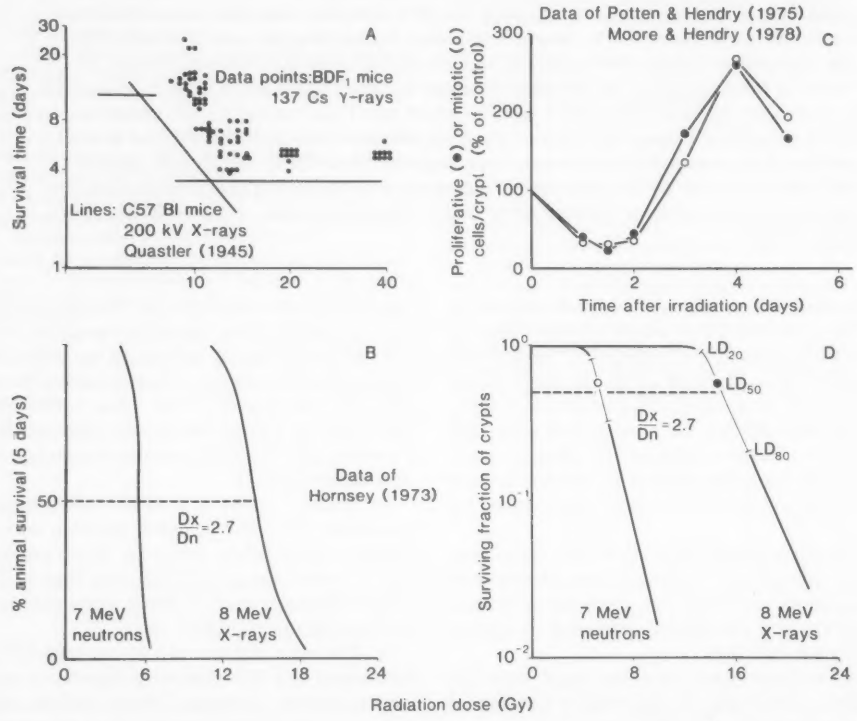

FIGURE 1.—Aspects of GI injury following whole-body irradiation. **A:** Survival time of mice as a function of radiation dose in Gy, with both axes on a logarithmic scale. Data points are for individual mice (J. V. Moore, unpublished data); lines are best fits to data given in ref 5. **B:** % of C3H mice surviving 5 days after single doses of x-rays or fast neutrons (ref 10). **C:** Proliferative activity in jejunal crypts of $B6D2F_1$ mice after irradiation by ^{137}Cs γ-rays (refs 8,9). **D:** Surviving fraction (log scale) of jejunal crypts in C3H mice 4 days after single doses of x-rays or fast neutrons. Surviving fractions corresponding to the whole-animal $LD_{50/5}$ doses have been marked on the crypt survival curves (ref 10).

9:00–10:00 AM, except for HN_2, which was injected either ip, or iv via the lateral vein of the tail.

Assays

(*a*) Groups of ten mice were injected with one of a range of drug doses, the lowest of which was chosen as being greater than the approximately determined $LD_{10/30\ day}$ doses for $B6D2F_1$ mice (BCNU, 38 mg/kg; HN_2, 2.75 mg/kg; CY, 250 mg/kg; ADR, 13 mg/kg; 5-FU, 190 mg/kg; and ACT D, 525 μg/kg). Animals were inspected three times daily between day of treatment and 30 days thereafter. The number of dead mice at each time was noted.

(*b*) Mice were injected with a single dose of one of the six drugs, and groups of three were killed at different intervals up to 4 days after treatment. Five-micron thick, transverse sections of jejunum were prepared and stained with hematoxylin and eosin, and the number of mitotic figures per column of crypt epithelial cells was scored. Thirty columns (ie, 30 half-crypts in 30 different crypts) were scored per animal.

(*c*) Groups of four to six mice were injected with one of a range of drug doses and killed 4.0 days later. Using transverse sections of jejunum, a "surviving fraction" of regenerating crypts was calculated relative to the number of crypts around the intestinal circumference in saline-injected controls (7,13).

(*d*) For comparison with the clonogenic response of the intestine, dose-response curves were obtained for primitive, clonogenic cells of the bone marrow (CFU_s), measured by the assay of Till and McCulloch (14). Graded, single doses of each drug were injected into groups of three animals at 9:00–10:00 AM. Survival of CFU_s was measured 24 hours later by injecting cell suspensions from the femurs of treated or control mice into recipients whose own marrow had been ablated by 8.5 Gy of 10-MeV electrons. The recipients were killed 8 days later and the number of colonies on the spleen was counted. Surviving fractions of CFU_s were calculated relative to numbers of colonies from saline-injected donors.

All experiments were repeated at least once, the data being pooled. Wherever possible, the different kinds of assay were run in parallel (ie, on the same day, using the same stock solution of drug, exactly age-matched mice, same personnel, etc). This was done to minimize the very large discrepancies in dose effects that can arise between drug studies done at different times (15).

Analysis of Results

Data on survival of animals versus dose of drug were analyzed by means of a "probit" computer program that fitted a curve to the data, using minimum chi-square techniques (16). The program calculates the LD_{50} dose ± 1 SE and measures the steepness of the dose-survival curve in terms of the "probit width," K, ie, the standard deviation of the probit curve, which is also the dose required to reduce survival from 84% to 50% or from 50% to 16%. Several authors have attempted to relate these survival curves for irradiated animals to those for populations of "tissue-rescuing cells," whose continued clonogenic capacity or sterilization is thought to determine the fate of the whole tissue or organism. We have assumed that some similar, quantitative, causal relationship exists between survival of cells and tissues after chemotherapy, and have used the model of Lange and Gilbert (17,18), which relates mortality probability for the animal (Pm) to parameters of mammalian cell survival curves by: probit (Pm) = $(D\text{-}LD_{50})/K = (D - Do.\ln(1.9\ N\alpha E))/1.2\ Do$, where D is the administered dose, N is the number of tissue-rescuing cells in the organism, α is the probability that one such cell will cause survival, E is the extrapolation number, and Do is the inverse of the final slope of a multitarget-type cell survival curve (the model assumed here for the response of the tissue-rescuing cells). Data on survival of intestinal crypts or of CFU_s have been analyzed using a modification of the above model (19).

RESULTS

Previous studies of whole-body irradiation with ^{137}Cs γ-rays showed that radiation-induced GI death in $B6D2F_1$ (Pat) mice occurred within the time range of 4–8 days (fig 1A; ref 12; and J. V. Moore, unpublished data). For all six drugs tested, some deaths occurred within 8 days (fig 2). However, only BCNU mimicked the classic radiation picture of survival times between 4 and 8 days over a relatively long range of doses (in this case, threefold). There was also a plateau for 5-FU, although at a later average time. For HN_2 and ADR, MST dropped slowly as a function of dose, falling within the 4–8-day band over an approximately twofold dose range. For CY and ACT D, MST decreased sharply with increasing dose, with no evidence of a plateau in the period of interest. MST was then calculated for all mice dying at or before 8 days, over a twofold range of drug doses. The lowest dose in each range was that at which deaths within 8 days were first seen. Between drugs, MST varied up to twofold, with CY and ACT D having mean values < 4 days (table 1).

From such survival data, the $LD_{50/8}$ (dose at which 50% of mice in a group died within 8 days of injection) and the Do derived from measurement of K were calculated. With the exception of 5-FU, the curves of mouse survival versus dose were relatively very steep, ie, Do < 15% of LD_{50}, and the calculated errors on the two parameters were correspondingly small (table 2). For CY, only two dose groups contained survivors besides those groups with 100% or 0% survival; in these circumstances, errors calculated by the program became vanishingly small and biologically meaningless. The cellular response of the small intestine to equitoxic ($LD_{10/8}$) doses of the six drugs

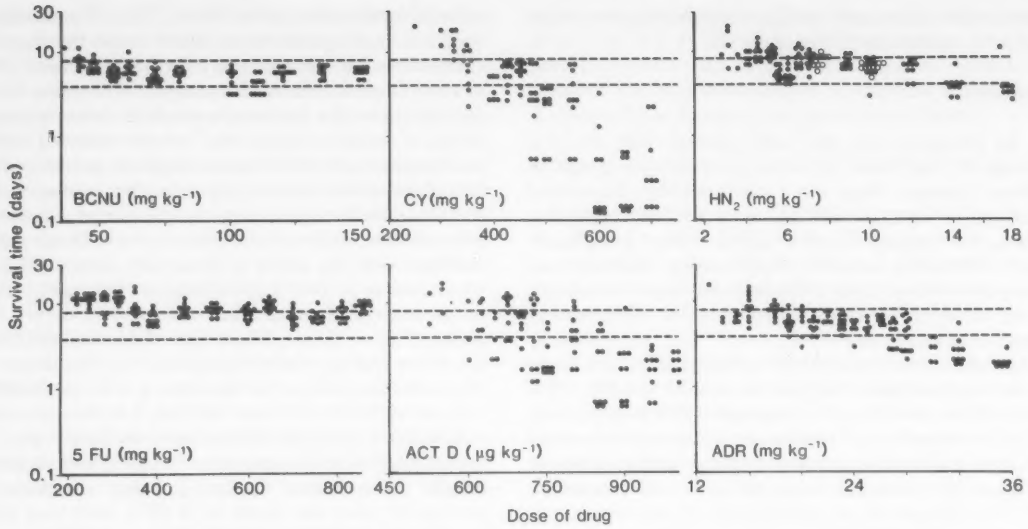

FIGURE 2.—Survival time (log scale) of individual mice treated by a single ip injection of chemotherapeutic drug. Dashed lines indicate times of 4 and 8 days. Open circles in HN_2 panel are for mice treated iv.

was measured in crypts of the jejunum. Suppression of mitosis occurred within a few hours; this suppression occurred more rapidly and completely for the three alkylating agents (HN_2, CY, and BCNU) than for the other three drugs (fig 3). However, recovery of mitosis to control levels or greater required the same 3-4 days for all drugs, as also for radiation (fig 1C).

Survival of clonogenic cells in crypts was measured over dose ranges that always contained the $LD_{50/8}$ dose. The shapes of curves of crypt survival versus dose varied widely between agents (fig 4). The doses that reduced crypt survival to 0.5 (D_{50}) and the Do doses are given in table 2. It was noted that: (*a*) For dose ranges that spanned the mouse $LD_{50/8}$ dose, neither CY nor ACT D caused *any* depletion of crypt number. (*b*) For the four agents that did ablate crypts, the surviving fraction of crypts at the $LD_{50/8}$ dose varied over a 17-fold range (0.06 for HN_2 to 1 for BCNU). (*c*) Altering the route of administration of HN_2 from ip to iv increased the $LD_{50/8}$ dose by a factor of 1.4 ± 0.1 and also increased the D_{50} dose, but by the significantly higher factor of 3.1 ± 0.7 (fig 5).

Regarding the response of CFU_s, it proved impossible to obtain a satisfactory reproducible curve with 5-FU. For the other three drugs that destroyed crypts, the final slopes of the CFU_s survival curves were similar to or even shallower than those for crypts (BCNU: Do = 27.5 ± 7.4 mg/kg; HN_2: Do = 2.50 ± 0.37 mg/kg; ADR: Do = 6.8 ± 0.5 mg/kg) (table 2). Of the two drugs that did not destroy crypts, the surviving fraction of CFU_s after ACT D reached a plateau at doses > 100 μg/kg. However, the CFU_s curve for CY could be fitted by a steep, exponential function (Do = 101 ± 7 mg/kg) over a two-log range of survival. When the route of administration of HN_2 was changed from ip to iv, the survival curve shifted in the

TABLE 1.—Mean survival time of mice dying within 8 days of single doses of cytotoxic drugs (twofold range) or of whole-body irradiation

Agent of assay	Dose range*	Mean survival time ± 1 SE in days for deaths on or before Day 8
CY	325-700	3.3 ± 0.2 (118)†
ACT D	525-1000 μg/kg	3.4 ± 0.2 (83)
ADR	14-36	4.9 ± 0.2 (89)
BCNU	42.5-80	5.9 ± 0.2 (68)
HN_2	3-6	6.2 ± 0.2 (33)
5-FU	283-600	7.1 ± 0.2 (23)
^{137}Cs γ-rays	10-14 Gy	6.5 ± 0.1 (76)

*Unless specified otherwise, values = mg/kg.
†No. of mice in each calculation is shown in parentheses.

TABLE 2.—Survival parameters of mice and intestinal crypts after single doses of cytotoxic drugs or whole-body irradiation

Agent of assay	Parameter	Values of parameters for* — Animal survival	(8 days)	Crypt survival	(4 days)	Crypt surviving fraction (4 days) at $LD_{50/8}$ dose
BCNU (mg/kg)	LD_{50} or D_{50}	44.4 ± 0.3	(37-70)	95.5 ± 2.5	(19-200)	1 ± 0.02
	Do	2.19 ± 0.32		31.0 ± 2.3		
CY (mg/kg)	LD_{50} or D_{50}	340	(200-450)	? > 450	(50-450)	1 ± 0.03
	Do	9.83		—		
HN_2 ip (mg/kg)	LD_{50} or D_{50}	5.74 ± 0.17	(2-12)	1.76 ± 0.39	(0.5-13)	0.06 ± 0.02
	Do	1.49 ± 0.17		1.44 ± 0.11		
HN_2 iv (mg/kg)	LD_{50} or D_{50}	7.82 ± 0.18	(3-10)	5.42 ± 0.41	(3-13)	0.18 ± 0.03
	Do	1.44 ± 0.18		1.56 ± 0.13		
ADR (mg/kg)	LD_{50} or D_{50}	16.5 ± 0.4	(12-19)	13.9 ± 1.6	(6-28)	0.44 ± 0.10
	Do	1.93 ± 0.42		14.4 ± 3.5		
ACT D (μg/kg)	LD_{50} or D_{50}	688 ± 11	(450-1000)	? > 825	(100-825)	1 ± 0.04
	Do	71.2 ± 11.5		—		
5-FU (mg/kg)	LD_{50} or D_{50}	633 ± 117	(184-850)	679 ± 34	(50-800)	0.53 ± 0.03
	Do	401 ± 227		483 ± 88		
^{137}Cs γ-rays or 10-MeV electrons (Gy)†	LD_{50} or D_{50}	11.1 ± 0.2	(9-15)	10.2 ± 0.1	(9-15)	0.35
	Do	1.25 ± 0.22		1.23 ± 0.08		

*Values in parentheses = dose range used.
†Data of Hendry et al (ref 12) for male $B6D2F_1$ (Pat) mice.

opposite direction to that for crypt survival (fig 5). A D_{50} could not be measured because of the "dip" that occurs in CFU_s surviving fraction in the 24 hours following treatment by any of a number of drugs (20). At a surviving fraction of 10%, the ratio of iv to ip doses of HN_2 was 0.39:1. The Do of the iv curve was 1.68 ± 0.33 mg/kg and that for the ip curve was 2.50 ± 0.37 mg/kg.

DISCUSSION

This paper has queried the validity of employing survival time as an indicator of a particular lethal syndrome when the characteristics of that syndrome have been established for one therapeutic modality but are being assumed for another. With respect to whole-body irradiation, the GI syndrome reflects the acute failure of renewing tissues within two organs, the mucosa of the intestine (small) and the hemopoietic tissues of the bone marrow (6). These renewing tissues have been referred to as "hierarchical," in that they contain a more or less discrete compartment of stem cells which have high replicative potential and are usually relatively radiation-sensitive, giving rise to daughter populations with less or no division capacity and relative radiation resistance (21,22). Survival time remains constant with dose even though mortality increases radically (figs 1A, 1B) because, although increasing numbers of stem cells are killed with increasing dose, survival time at all lethal doses reflects the longevity of pre-existing, radiation-resistant, functional cells. The superimposition of a further syndrome is often inferred from changes in MST, eg, the descending portion in figure 1A where an increasing proportion of animals are dying from the "GI syndrome" rather than the "hemopoietic syndrome."

Three questions arise when comparing early death following radiation and cytotoxic drugs. First, and relatively trivial, is the use of such a broad criterion of comparison as "first-week" mortality appropriate? Second, even when survival times are closely comparable, are the underlying phenomena quantitatively similar? Third, confidence in the relationship of radiation-induced, first-week mortality with GI injury rests partly on the continued correlation of effect when total absorbed dose is altered by changing radiation quality, fractionation schedule, dose rate, etc (10); can the same manipulations be performed to achieve a similar effect with cytotoxic drugs?

In the present study, we noted that MST varied between agents and that for three and possibly four out of six drugs, MST was an inverse function of dose over the

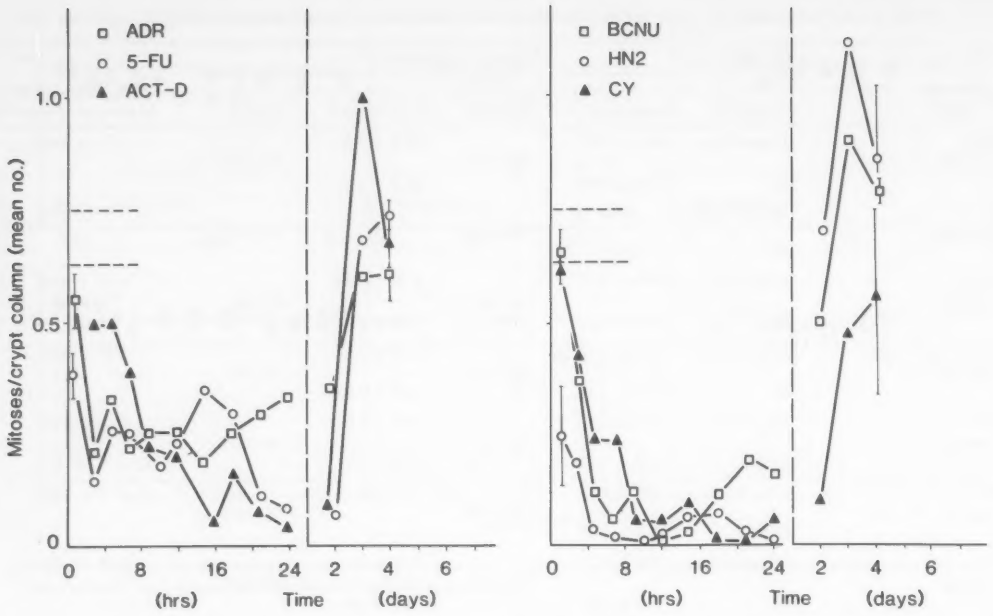

FIGURE 3.—No. of mitoses/crypt column (cell positions 1-21) at different intervals after 40 mg/kg of BCNU, 325 mg/kg of CY, 3.5 mg/kg of HN_2, 13.5 mg/kg of ADR, 550 μg/kg of ACT D, or 200 mg/kg of 5-FU. Horizontal dashed lines delimit ± 1 SE of the mean No. of mitoses in untreated animals killed at various times during the 24-hr cycle. For clarity, error bars (1 SE) are shown only on the 1- and 96-hr data points.

range measured. To identify all these deaths with GI toxicity would require (*a*) that the dynamics of expression of injury in this organ vary between agents, and/or (*b*) that with CY and ACT D in particular, these dynamics of injury are dose-dependent. There is little evidence for proposition (*a*). For all six drugs and for radiation, inhibition and recovery of mitosis occurred over the same 4-day time scale (figs 1C, 3). The incidence and time scale of cellular necrosis were also invariant between these drugs (23). The distribution of dead cells along the crypt varies somewhat between agents, but for all the drugs described here, damage is initially confined to the lower half of the crypt, with no direct lethal injury to cells on the villus (23). Regarding dose dependence, Devik and Hagen (24) investigated the histopathological effects on the murine small intestine of five drugs, including CY and HN_2, using between three and four dose sizes of each. Although the absolute values of the nadir of cell number or peak number of pyknotic cells varied between doses in some cases, the time scale did not. This was also true for ADR (25).

The alternative is to consider that MST changed with dose because of the intervention of other toxic effects with higher thresholds for overt expression of damage but shorter times of onset, as with GI and marrow deaths after irradiation. Berenbaum (26) showed that high doses of CY in combination with potassium iodide caused massive pleural effusion and pulmonary edema within a few hours of injection, possibly directly but perhaps as a consequence of myocardial insult. The great majority of these BALB/c mice died within 24 hours. Using CY alone, doses > 600 mg/kg were required to produce this rapid cardiopulmonary death. We have observed the rapid onset of death at a dose of 475 mg/kg of CY alone (fig 2). As the CY $LD_{50/30}$ for BALB/c mice was 450–500 mg/kg and for our $B6D2F_1$ mice only 300 mg/kg, it may be that in the latter strain all modes of death are expressed at lower administered doses. For ACT D, Philips et al (27) reported an $LD_{50/14}$ (ip) of 760 μg/kg in H_A/ICR mice, compared to 620 μg/kg in the present study. In both cases, the incidence of deaths within 48 hours of injection was high. Philips et al (27) commented on the rapid rise in hematocrit and fall in body weight of rats given ACT D and speculated on whether a form of "early damage" to intestinal mucosa might permit penetration of enteric toxins. However, as we have noted, the dynamics of histopathological intestinal injury in mice given ip ACT D differed little from those of other drugs. For ACT D and CY at least, it seems improbable that first-week mortality is determined by a GI syndrome.

For three drugs (HN_2, BCNU, and 5-FU), MST was similar to that after irradiation (table 1). However, only

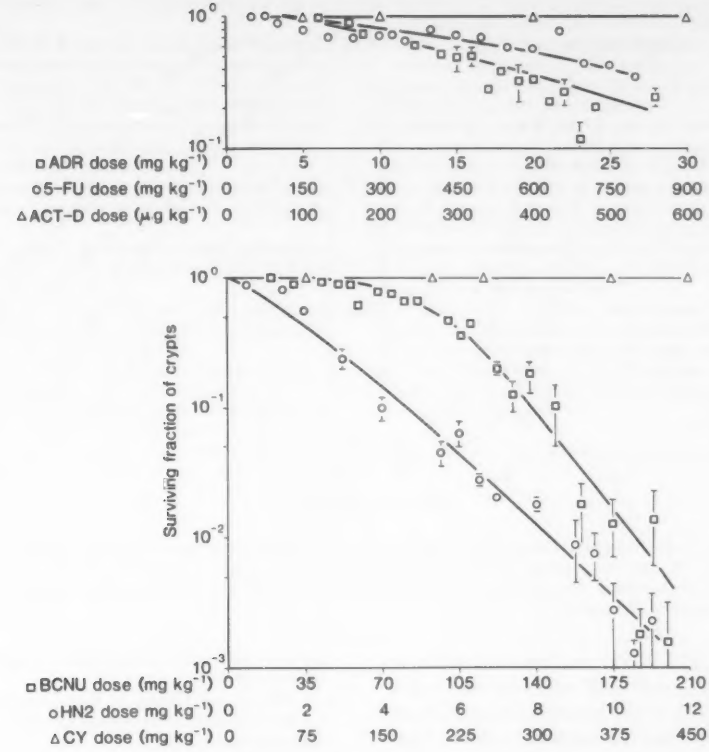

FIGURE 4.—Surviving fraction of intestinal crypts 4 days after graded single doses of drugs. Error bars as ± 2 SE. Reproduced by permission of the publishers of the *British Journal of Cancer*.

for 5-FU were both the D_{50} *and* the Do values of animal and crypt survival curves closely comparable (table 2). Using BCNU (and also ADR), the Do derived from the survival curve for animals was much steeper than that for either crypt microcolony-forming cells or marrow CFU_s. This inequality might arise because (*a*) survival of the animal is determined by cells in organs other than those we have examined here, ie, again by syndromes other than the GI; (*b*) assuming the lethal syndrome to be GI, the actual tissue-rescuing cells are much more sensitive than the microcolony-forming or CFU_s compartments of the respective tissues; or (*c*) the relationships of the "administered" dose, which is used as the independent variable of survival curves, to the "absorbed" or "effective" dose differs for different tissues and/or endpoints.

The CFU_s are not thought to be the ultimate stem cell of the hemopoietic tissues (28), while the numbers of microcolony-forming cells in each intestinal crypt do not match the numbers of pluripotent stem cells measured by histological techniques (22,29). Lacking a firm identification and a clonogenic assay for these true stem cells in marrow and gut, argument (*b*) above is conjectural. Undoubtedly, there are cell populations in these and other tissues that have exceptionally steep in vivo survival curves after cytotoxic drugs, eg, 17.5 mg/kg of CY for marrow colony-forming cells grown in diffusion chambers in mice (30), or 1.33 mg/kg of ADR for spermatogenic stem cells of the murine testis (ref 31; *see* values for mouse survival in table 2).

The relationship of chemotherapeutic dose to biological effect is complex. In experimental studies of GI toxicity after drugs or drugs plus radiation, drug dose has been administered by the ip route almost exclusively (32). In the present experiments, the $LD_{50/8}$ dose for HN_2 was varied by altering the route of administration to iv. The resultant shifts in curves of survival for animals, microcolony-forming cells, and CFU_s were all different (fig 5). The same administered dose given ip will often cause more abdominal toxicity than when given iv or sc because, for example, ip injections can result in peritoneal adhesions and peritonitis as well as in direct mucosal cy-

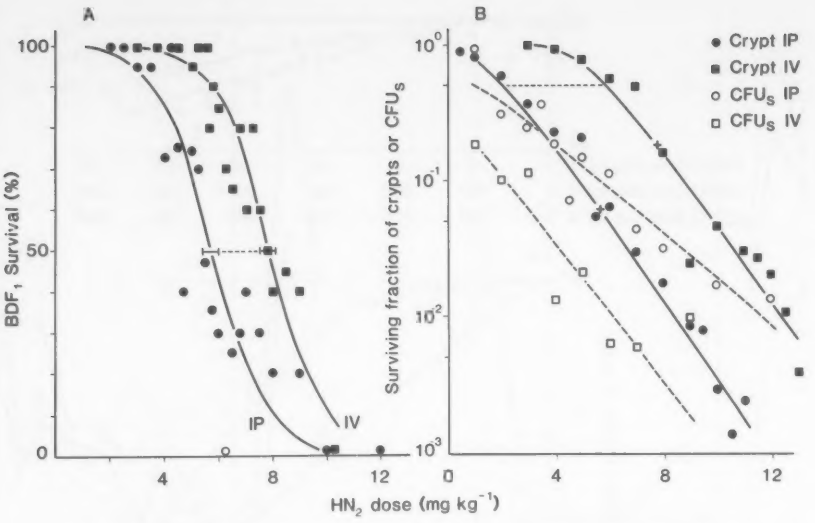

FIGURE 5.—**A**: Proportion of animals surviving > 8 days after graded single doses of HN_2, administered either ip or iv. **B**: Surviving fraction of intestinal crypts (solid lines) or of bone marrow CFU_S (dashed lines) after graded single doses of HN_2, given ip or iv. Crosses on crypt curves are the surviving fractions corresponding to the respective $LD_{50/8}$ doses.

totoxicity, eg, after ACT D (27) or ADR (33). We have also demonstrated "hot" and "cold" spots for survival of crypts on opposite sides of an intestinal circumference treated by ip HN_2 (34). This did not occur after iv injection and was attributed to direct absorption of drug across the serosa to the mucosa. Given these differences, and particularly if the lethal syndrome is a result of injury to several tissues, it is perhaps not surprising that good quantitative agreement was not obtained between the ratio of ip and iv doses for lethality and for any *one* tissue endpoint.

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Thrombocytopenia After Combination Therapy With Aminoglutethimide and Tamoxifen: Which Drug Is To Blame?[1]

Joseph Ragaz,* Noel Buskard, and Mohamed Manji[2]

Because of the theoretical rationale of the benefit of combined aminoglutethimide and tamoxifen in the treatment of breast cancer, we have treated patients with metastatic breast cancer with both agents given together. Some of our observations on this treatment and its side effects have been presented elsewhere (1,2). We report here in more detail a case of thrombocytopenia developing in one patient 7 weeks after the combined hormone therapy was started.

Tamoxifen can cause thrombocytopenia in 2%-5% of patients (2-4). Glutethimide (Doriden), a sedative chemically related to aminoglutethimide, has also been reported to cause thrombocytopenia (5). However, at the time our patient was treated, thrombocytopenia had been reported infrequently with aminoglutethimide. Therefore, we attempted to establish which of these two drugs caused the platelet suppression in our patient.

CASE REPORT

A 53-year-old woman with carcinoma of the breast with bone metastases was previously treated with mastectomy, radiotherapy to the pelvis and lumbar spine, and chemotherapy with doxorubicin and cyclophosphamide. On May 13, 1979, she began a regimen of medical adrenalectomy (aminoglutethimide, 1 g/day; hydrocortisone, 40 mg/day) in combination with tamoxifen (10 mg twice/day orally). On the date the combination of aminoglutethimide and tamoxifen was started, the cbc was normal, with a platelet count of 220,000/mm^3. Seven weeks later, the patient was admitted to the emergency room because of a sudden onset of dizziness, weakness, sweating, and passing large amounts of bright red blood from the rectum. The blood pressure was 80/0 mm Hg and the pulse was 135/minute and regular. The Hgb was 5.2 g/100 ml, the wbc count was 10,000/mm^3, and the platelet count was 13,000/mm^3. The patient was treated with 6 units of blood, 8 units of platelets, normal saline infusion, and hydrocortisone iv. Both aminoglutethimide and tamoxifen were stopped. Hydrocortisone was continued at 100 mg/day. The investigations showed normal blood chemistry, including prothrombin time, partial thromboplastin time, and fibrin degradation products. The patient's bone marrow aspirate showed normal cellularity, adequate number of normal megakaryocytes, and occasional cells consistent with breast carcinoma.

One week following admission, the patient's platelet count increased to 120,000/mm^3. Three weeks later, while asymptomatic with an Hgb of 13.4 g/100 ml, a wbc count of 6400/mm^3, and a platelet count of 250,000/mm^3, aminoglutethimide was restarted at 500 mg/day (50% reduction), tamoxifen at 10 mg/day (50% reduction), and hydrocortisone at 60 mg/day. Three weeks later, the patient noticed red blood spotting in the stool. In the emergency room that day, she was normotensive with an Hgb of 9 g/100 ml, a wbc count of 5300/mm^3, and a platelet count of 8000/mm^3. She was transfused with 8 units of platelets, aminoglutethimide and tamoxifen were stopped, and hydrocortisone was continued at 40 g/day. A repeat bone marrow aspiration showed normal cellularity, normal megakaryocytes and, this time, no cancer cells were seen. With no further treatment, the patient remained asymptomatic; 5 days later the Hgb was 12.1 g/100 ml, the wbc count was 6100/mm^3, and the platelet count was 150,000/mm^3. Tamoxifen-induced thrombocytopenia was suspected and, therefore, it was not subsequently resumed. Aminoglutethimide (1.0 g) was restarted and hydrocortisone was continued at 40 mg/day.

Ten days later on August 13, 1979, the patient had an Hgb of 11.1 g/100 ml, a wbc count of 5000/mm^3, and a platelet count of 11,000/mm^3. Aminoglutethimide was stopped. Six days later, the platelet count increased to 150,000/mm^3, recovering without platelet transfusions. On September 12, 1979, tamoxifen (10 mg twice/day orally) was restarted. While the patient was receiving tamoxifen during the months of October, November, and December 1979, her platelet count remained > 150,000/mm^3.

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[2]Cancer Control Agency of British Columbia and the Faculty of Medicine, University of British Columbia, Canada.

* *Reprint requests to:* Dr. Joseph Ragaz, Division of Medical Oncology, Cancer Control Agency of British Columbia, 2656 Heather St, Vancouver, British Columbia, V5Z 3J3 Canada.

DISCUSSION

Thrombocytopenia due to aminoglutethimide has been reported on several occasions (6-8). Because of the possibility of massive bleeding due to thrombocytopenia, as seen in our patient, cbc and platelet count should be checked in the initial few weeks in patients receiving aminoglutethimide. We have not seen any further cases among 141 patients treated with aminoglutethimide since 1977 (incidence of severe thrombocytopenia with bleeding: < 0.6%). Two other patients developed thrombocytopenia in connection with aminoglutethimide; in both patients, however, the platelet count did not drop to < 60,000/mm^3 (nadirs, 65,000 and 80,000/mm^3 in the first and second patients, respectively). Both patients remained asymptomatic without signs of bleeding; their platelets promptly returned to normal after discontinuation of aminoglutethimide. Therefore, three of 141 patients treated with aminoglutethimide developed thrombocytopenia (incidence of 2.1%).

The initial impression that the patient had tamoxifen-induced thrombocytopenia led us to initiate a second trial of aminoglutethimide. The proof that tamoxifen was not causal comes from the tolerance of tamoxifen and the normal platelet count after aminoglutethimide was discontinued. At present, the mechanism of thrombocytopenia due to aminoglutethimide is not clear; immune mechanisms with peripheral destruction of platelets are suspected. This possible mechanism is supported by the normal bone marrow appearance of megakaryocytes and positive direct platelet antibody tests observed in our patient on one occasion. Decreased bone marrow reserve due to past chemotherapy, radiotherapy, or carcinoma invading the bone marrow may be of additional significance.

An awareness of thrombocytopenia occurring with aminoglutethimide treatment may increase the recognition of this complication and allow for appropriate early discontinuation of drug, which in turn may improve the safe use of this useful hormone modality treatment for metastatic breast cancer.

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Aggressive Doxorubicin-Containing Regimen (Prednisone, Methotrexate, 5-FU, Doxorubicin, and Cyclophosphamide; PM-FAC) in Disseminated Estrogen Receptor-Negative Breast Cancer[1]

Joanne Mortimer,[2,3,*] Robert B. Livingston,[2,3] Ina J. Hardesty,[2] Carl W. Groppe, Jr,[2] Joseph D. Purvis, III,[2] Manjula Gupta,[2] and Nancy Flournoy[4]

Twenty-seven women with disseminated estrogen receptor-negative breast cancer received an aggressive chemotherapy program of prednisone, methotrexate, 5-FU, doxorubicin, and cyclophosphamide. Responses were achieved in 21 of 26 (81%) evaluable patients, eight (31%) of whom had complete responses. The median survival was 17 months. Despite the favorable overall response and a significant number of complete responses, all patients eventually relapsed. Although most patients relapsed systemically, two relapsed initially in the CNS. [Cancer Treat Rep 68:1017–1018, 1984]

In women with breast cancer, a negative estrogen receptor (ER) defines a clinical course distinctively more aggressive than that of ER-positive disease. Characteristically, these patients have a shorter disease-free interval, tend to develop visceral metastases, have a shortened survival after dissemination, and possibly have a poorer response to conventional chemotherapy (1–3).

From 1980 to 1982 at the Cleveland Clinic Foundation, we treated 27 patients with disseminated ER-negative breast cancer with an aggressive doxorubicin-containing regimen (prednisone, methotrexate, 5-FU, doxorubicin, and cyclophosphamide; PM-FAC).

MATERIALS AND METHODS

Patients with biopsy-proven disseminated breast cancer were eligible if their ER was quantitated as ≤ 3 fmols/mg. All patients had objectively measurable disease by physical examination, chest x-ray, and liver-spleen or bone scan. Bone scan was not the sole measurement of metastatic disease in any patient. A bone marrow aspirate and biopsy were performed in patients with elevations of serum LDH or whose peripheral smears were compatible with marrow involvement. Response was defined by standard criteria of the Southwest Oncology Group.

Treatment was divided into inpatient induction therapy and outpatient maintenance therapy. In the hospital, after oral prednisone at 150 mg/day for 3 days, patients received methotrexate (40 mg/m^2) followed immediately by a 72-hour infusion of 5-FU (800–1200 mg/m^2/day). Doxorubicin (40–60 mg/m^2) was given over a 24–48-hour period during the 5-FU infusion, either as a continuous infusion into a central venous line (nine patients) or in four divided doses over 48 hours (17 patients). Cyclophosphamide (500 mg/m^2) with hydrocortisone (1 g) was given at the end of the 5-FU infusion. Patients who had received extensive prior radiation therapy were given the lower doses of 5-FU and doxorubicin. Following four cycles of induction therapy, patients received a monthly maintenance program of prednisone (80 mg/day for 5 days), cyclophosphamide (1 g/m^2), and methotrexate (40 mg/m^2). Maintenance was discontinued at 1 year or with evidence of progressive disease.

RESULTS

The clinical characteristics of the patients receiving PM-FAC are summarized in table 1. Of the 27 women treated with PM-FAC, 26 were evaluable for response, since one patient died within 1 month of therapy. Eight patients (31%) obtained a complete response, with an additional 13 (50%) achieving a partial response. Thus, an overall response rate of 81% was seen in the PM-FAC-treated women. Response rates were independent of age, prior therapy, menopausal status, site or timing of ER de-

[1]Received Apr 29, 1983; revised Feb 7, 1984; accepted Mar 1, 1984.
[2]Cleveland Clinic Foundation, OH.
[3]University of Washington, Seattle.
[4]Fred Hutchinson Cancer Research Center, Seattle, WA.
[*]*Reprint requests to:* Joanne Mortimer, MD, University of Washington, NN111 University Hospital, RC-08, 1959 NE Pacific St, Seattle, WA 98195.

TABLE 1.—Clinical characteristics of patients receiving PM-FAC

Characteristic	No. of patients (%)
Age in yrs (range): 48 (33-79)	
Total	27
Premenopausal	16
Disease-free interval (mos): 12	
Prior adjuvant therapy*	6
CMF	3
Melphalan	2
CMF-FAC	1
Prior therapy for disseminated disease	4
Metastatic sites	
CNS	2 (7)
Lung (nodules)	7 (26)
Lung (lymphangitic)	3 (11)
Liver	7 (26)
Bone marrow	14 (52)
Soft tissue and/or bone only	2 (7)
Median No. of metastatic sites: 2.0	
≥ 3 sites of metastasis	12 (44)

*CMF = cyclophosphamide, methotrexate, and 5-FU.

termination, disease-free interval, or number of metastatic sites. No difference in response was detected between patients who received doxorubicin by continuous infusion and those who received divided doses. The median survival was 17 months in the PM-FAC-treated group.

The most commonly encountered toxic effect was mucositis, which occurred in 12 (44%) patients. Mucositis in two patients was classified as life-threatening with ulceration and bleeding. In one instance, the patient was receiving simultaneous irradiation to the cervical spine. Thirty percent of the patients developed life-threatening neutropenia, defined as a total granulocyte count $< 500/mm^3$. One patient experienced severe thrombocytopenia (platelet count $< 50,000/mm^3$) without hemorrhagic complication. Two patients died from myelosuppressive complications: one with sepsis and one with sepsis and liver failure.

At last follow-up, 19 of 21 responding patients had relapsed 4-12 months after initiation of therapy. Four patients relapsed in the CNS: two with parenchymal lesions and two with leptomeningeal spread. In the two patients with leptomeningeal carcinomatosis, the CNS was the initial site of recurrence and occurred prior to the development of other systemic disease.

DISCUSSION

The PM-FAC regimen was designed to optimize chemotherapy dosage and timing. Prednisone was used for its reported G_1-S arrest. Methotrexate was given prior to 5-FU to take advantage of schedule-dependent synergy. Infusion 5-FU is possibly associated with higher response rates than bolus 5-FU and clearly is less myelosuppressive (4). Doxorubicin may be the single most active agent in the therapy for ER-negative breast cancer with respect to both response and overall survival (5).

The response rate to PM-FAC is superior to that of other doxorubicin-containing regimens in ER-negative breast cancer (5). The median time from the diagnosis of breast cancer to parenchymal spread is 23 months (6) and to leptomeningeal carcinomatosis is 25 months (7). More than one-half of our patients lived long enough to be at risk for the development of CNS disease. Two of our patients developed parenchymal metastases and two developed leptomeningeal spread. The leptomeningeal disease was the initial site of relapse in these latter two patients. In planning aggressive chemotherapy programs for ER-negative breast cancer, consideration should be given for inclusion of CNS prophylaxis.

Even with the aggressive chemotherapy employed in the PM-FAC regimen, no patients were "cured" of their disease. Future studies attempting to improve our results might include more aggressive maintenance therapy. Since doxorubicin is such an active agent in ER-negative breast cancer, it could be included throughout induction and maintenance therapy until a maximum cumulative dose has been reached. An alternative approach might include intensive consolidation therapy. We have initiated such a study in women with disseminated ER-negative breast cancer. Following induction chemotherapy, women achieving a complete or partial response are "consolidated" with either sequential hemibody irradiation or high-dose chemotherapy and total-body irradiation with autologous bone marrow infusion.

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Multidrug Chemotherapy (Vincristine, Bleomycin, and Methotrexate [VBM] With Radiotherapy in Stage III–IV Squamous Cell Carcinoma of the Head and Neck[1]

Riccardo Rosso,[2,*] Marco Merlano,[2] Mario Roberto Sertoli,[2] Elisabetta Campora,[2] Daniele Scarpati,[3] Filippo Borasi,[4] and Eugenio Pallestrini[4]

Thirty-three patients with stage III–IV squamous cell carcinoma of the head and neck previously untreated with chemotherapy or radiotherapy were given a multidrug cytotoxic regimen, VBM (vincristine, bleomycin, and methotrexate), in combination with radical radiotherapy. Of the 32 evaluable patients, 11 (34.4%) achieved clinical complete response with negative biopsy, 15 (46.8%) achieved partial response, three (9.4%) had no change, and three (9.4%) had disease progression. At 30 months, the actuarial survival rate for complete responders with negative biopsy was 62.2% and the overall survival rate was 30.8%. Hematologic and gastrointestinal toxicity was moderate, but one patient developed life-threatening mucositis and one patient died due to acute renal failure. Our data do not confirm prior more favorable results obtained with the same treatment. [Cancer Treat Rep 68:1019–1021, 1984]

Treatment consisting of surgery and/or radiation therapy for patients with squamous cell carcinoma of the head and neck has frequently been successful in earlier stages of disease, but when the same therapeutic modalities are applied to more advanced head and neck cancer, results are generally poor. The 5-year survival rate for stage III-IV cancer is still ⩽ 25%. Traditionally, chemotherapy has been used for palliation of recurrent disease but theoretically the administration of chemotherapy prior to definitive radiotherapy and/or surgery could be advantageous since a combined modality approach could improve local control and prevent distant metastasis (1). In fact, results from various combined modality trials have been promising (2,3). O'Connor et al (4) reported a therapeutic gain in stage III-IV disease when combination chemotherapy (VBM: vincristine, bleomycin, and methotrexate; the latter drug was administered by 24-hour infusion) was given synchronously during radical radiotherapy. This report prompted us to conduct a study to verify the effectiveness of the above combined modality treatment. We administered VBM synchronously with radiotherapy in patients with stage III-IV squamous cell carcinoma of the head and neck previously untreated with chemotherapy or radiotherapy. In our trial, the VBM regimen was modified only in that methotrexate was given iv in 2 hours to allow for outpatient treatment.

MATERIALS AND METHODS

Between August 1979 and December 1981, 33 patients (28 with histologically proven, untreated, inoperable stage III-IV squamous cell carcinoma of the head and neck and five with recurrent disease after primary) were entered in our trial. Thirty-one patients were male and two were female, with a median age of 58 years (range, 37–75) and a median Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1 (range, 0–2). At the time of entry in the study, ten patients (nine untreated and one with recurrent disease) had regional lymph node involvement. Pretreatment evaluation included: history and physical examination; cbcs; blood glucose; urea; creatinine; electrolytes; liver function tests; urinalysis; chest x-ray; ear, nose, and throat examination; and liver scan. VBM was administered according to the following schedule: Day 1 (0 hour), vincristine (1.2 mg/m^2 iv) and (6 hours), bleomycin (30 mg iv); Day 2 (24 hours), methotrexate (200 mg iv in 1000 ml/2-hour infusion); Day 3 (48

[1] Received May 12, 1983; revised Feb 16, 1984; accepted Mar 1, 1984.

[2] Istituto Nazionale per la Ricerca sul Cancro, Istituto di Oncologia dell'Università, Genova, Italy.

[3] Istituto di Radiologia dell'Università, Genova, Italy.

[4] Divisione di Otorinolaringoiatria, Ospedali Civili, Genova, Italy.

[*] *Reprint requests to:* Riccardo Rosso, MD, Department of Chemotherapy, Istituto Nazionale per la Ricerca sul Cancro, V.le Benedetto XV, 10, 16132, Genova, Italy.

hours), leucovorin (12 mg orally followed by 12 mg orally every 6 hours for three doses); and Day 4 (72 hours), radiation therapy was started. Radiotherapy (Cobalt-60) was given employing parallel opposed field, and treatment fields included the primary tumor and lymph node chains on both sides of the neck. Dosage schedule consisted of 2 Gy 5 times/week for 2 weeks. VBM was repeated after the second week of radiotherapy. The patients were given three courses of combined VBM and radiotherapy. Clinical response was evaluated according to International Union Against Cancer criteria. In addition, when clinical complete response was achieved, biopsy was obtained. If biopsy was negative, no additional treatment was given. On the other hand, when biopsy was positive the patient was considered a partial responder. Patients classified as partial responders either after biopsy or on the basis of clinical response had three additional courses of VBM only. Patients with stable or progressive disease were given alternative treatment.

RESULTS

Of the 33 patients entered, only 32 are evaluable, since one patient developed life-threatening mucositis after the first course of chemotherapy and the treatment regimen was discontinued. Eleven patients (34.4%) achieved a complete response with negative biopsy, 15 (46.8%) achieved a partial response, three (9.4%) had no change, and three (9.4%) had disease progression. Overall response and response according to disease stage, site, tumor differentiation, PS, and lymph node involvement are shown in table 1. The small number of patients in our series precludes any statistical analysis of differences in response rate attributable to any of the above factors. At 30 months after the start of the trial, median times to progression were 15+ months (range, 2+ to 30+) and 6+ months (range, 3+ to 30+) among complete and partial responders, respectively. Median survival was 22+ months (range, 2+ to 30+) among complete responders and 13+ months (range, 4-40+) among partial responders. Survival was significantly better in patients who responded to treatment since, both in patients who had no change or disease progression, median survival was only 6+ months (range, 5-6+). The overall survival rate was 30.8%, whereas the survival rate was 62.2% for complete responders with negative biopsy. Toxic effects of treatment were observed in only nine of 33 patients (27%). Hematologic toxicity was mild: no patient had a wbc count < 3000/mm^3 and only one patient had a platelet count < 100,000/mm^3. Moderate nausea and vomiting occurred in four patients, fever (38.5°C-40.0°C) occurred in five, and mucositis occurred in seven. In one of the seven patients developing mucositis, severe life-threatening symptoms were observed during the first course of chemotherapy, 2 days after methotrexate administration. Of the remaining six patients, mucous membrane toxicity generally occurred after the second chemoradiotherapy course. No patient required hospitalization and treatment was resumed only when symptoms were resolved, which caused a 7-14-day delay in treatment. We observed one (3%) toxic death among 33 patients: this patient, a partial responder, died of acute renal failure after his sixth dose of methotrexate. A second patient, again a partial responder, died of myocardial infarction 10 months after entry in the study. There was no evidence

TABLE 1.—Overall response and response to treatment according to disease site and stage, tumor differentiation, PS, and lymph node involvement (32 evaluable patients)*

Parameter	No. of patients	Response
Overall response		
CR	11 (34.4%)	
PR	15 (46.8%)	
NC	3 (9.4%)	
P	3 (9.4%)	
Site and stage†		
Nasopharynx		
III	2	2 CR
IV	1	1 PR
RD	0	—
Oral cavity		
III	3	2 PR, 1 NC
IV	4	3 PR, 1 P
RD	2	1 PR, 1 P
Oropharynx		
III	8	5 CR, 1 PR, 2 NC
IV	2	1 CR, 1 PR
RD	0	—
Hypopharynx		
III	4	1 CR, 2 PR, 1 P
IV	2	2 PR
RD	0	—
Larynx		
III	0	—
IV	1	1 PR
RD	3	2 CR, 1 PR
Tumor differentiation		
Differentiated	30	10 CR, 15 PR, 2 NC, 3 P
Undifferentiated	2	1 CR, 1 NC
PS (ECOG criteria)		
0	4	2 CR, 2 PR
1	25	9 CR, 12 PR, 3 NC, 1 P
2	3	1 PR, 2 P
Lymph node involvement		
Negative	23	9 CR, 11 PR, 3 NC
Positive	9	2 CR, 4 PR, 3 P

*CR = complete response (with negative biopsy); PR = partial response; NC = no change; and P = disease progression.

†Stage III ($T_{1-2}N_1$, T_3N_{0-1}) and stage IV ($T_{1-4}N_{2-3}$, N_4 N_0). RD = recurrent disease.

of disease progression at the time of death in either patient.

DISCUSSION

At 60 months, O'Connor and co-workers (5) observed a complete response rate of 83% and an overall survival rate of 41% when chemotherapy was given simultaneously with radiotherapy in advanced head and neck cancer. In contrast to these data, we observed a complete response rate of only 34.4% and at 30 months an overall survival rate of 30.8% with a very similar treatment regimen. In addition, the toxicity encountered in our series (3% toxic deaths) was less than could be expected on the basis of the above report (7.5% toxic deaths). The same investigators also had to abandon administration of VBM for more than four courses because of severe mucositis, and enteral feeding was required in about one-third of the patients. In our series, no severe toxic reactions were observed when, in partial responders, VBM was continued for up to a total of six courses. Furthermore, no patient required hospitalization or an unusual degree of supervision and all therapy could be administered on an outpatient basis. This discrepancy in results is difficult to explain, since our only modification of the original treatment regimen consisted of administering methotrexate in 2 hours and not in a 24-hour infusion. Very encouraging results recently obtained with the simultaneous use of a cisplatin-containing regimen and irradiation in advanced head and neck cancer (6,7) make such combined modality treatments attractive candidates for further trials.

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Chemotherapy for Advanced Epidermoid Carcinoma of the Esophagus With Single-Agent Cisplatin: Final Report on a Southwest Oncology Group Study[1,2]

Frank J. Panettiere,[3] Lawrence P. Leichman,[4] Eugene J. Tilchen,[5] and T. Timothy Chen[6,*]

Because of favorable experience with an outpatient cisplatin program in the management of patients with epidermoid carcinoma of the head and neck (1) and because a patient in that particular study had regression of an esophageal primary, the Southwest Oncology Group tested the program for patients with esophagus primaries. A preliminary report, demonstrating beneficial results among 15 fully evaluable patients, was published (2). This present report describes the experience of all 59 patients entered in the study.

Eligibility requirements included histologic proof of epidermoid carcinoma of the esophagus. Prior use of other chemotherapy agents was permissible. Patients were required to have an absolute granulocyte count $\geq 2000/mm^3$, a platelet count $\geq 150{,}000/mm^3$, a BUN and serum creatinine ≤ 20 and 1.6 mg/dl, respectively, and two functioning kidneys. Patients were required to have objectively proven residual tumor and were eligible whether or not it could be followed clinically. Those patients with measurable tumors were evaluated for response and survival; those with nonmeasurable disease were evaluated for survival duration alone.

Cisplatin at a dose of 50 mg/m^2 dissolved in 1000 ml of dextrose and one-half normal saline was administered over 30 minutes on the first and eighth day of each course. Subsequent courses, with doses modified based on prior toxicity, were begun every 28 days, or as soon thereafter as the renal function permitted (1,2).

Complete response was defined as the disappearance of all known tumor for at least 30 days. Partial response was defined as at least a 50% decrease in the sum of the products of the perpendicular diameters of all measurable tumor for at least 30 days. Stable disease was defined as at least 90 days without tumor growth. All other patients with measurable disease were categorized as having increasing disease. To compare different survival curves the generalized Wilcoxon test due to Gehan was utilized (3).

Two of the 59 patients entered were not eligible because of inadequate renal function. Forty-five patients had measurable disease and 12 had nonmeasurable, but known residual, disease. Twenty-six of the 57 patients had a poor pretreatment performance status (Southwest Oncology Group scale, 2-4; Karnofsky performance score, 10-60).

Of the 45 patients with measurable disease, 35 were judged to be fully evaluable for response. Reasons that the other ten patients might be called partially evaluable include death due to tumor, not toxicity, within 30 days in the study (six patients); toxicity which prevented a second course (two); and refusal to continue in the protocol for 30 days (two). In all, three patients had complete tumor regressions, six had partial regressions, and four had stable disease for at least 90 days. Of the 22 patients with increasing disease, one, who met measurement criteria for a partial response, is not so categorized. This is because he died before 30 days had elapsed, due to problems antedating his tumor. The nine patients with complete or partial responses constitute 25.7% of the fully evaluable, or 20% of all eligible, patients with measurable disease.

What we have characterized as responses had clear objective regressions. One complete responder had multiple nodal metastases above and below the diaphragm demonstrated at thoracoabdominal surgery. They were again demonstrated on computerized tomographic (CT) scan done 1 month later, when protocol chemotherapy began. Two months later a repeat CT scan showed marked regression. A repeat CT scan at 5 months showed no adenopathy. A surgical restaging at 8 months showed fibrotic residua and no histologically demonstrable tumor. A subsequent CT scan at 11 months showed no evident tumor and the clinical complete regression continues. The other two complete responders have already been described (2). One responder had a biopsy-proven primary lesion on esophagoscopy. Multiple repeat endoscopic biopsies have

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[3]University of Arkansas for Medical Science, Little Rock.
[4]Wayne State University Medical Center, Detroit, MI.
[5]Henry Ford Hospital, Detroit, MI.
[6]Biostatistical Center, Houston, TX.
[*]*Reprint requests to:* Southwest Oncology Group (SWOG-7806), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

all been histologically negative. His complete regression lasted 23 months, when tumor recurred. The other patient's measurable disease consisted of distinct nodules on chest radiographs. They shrunk to within partial response criteria and completely disappeared for 2 more months before the patient died, in clinical complete remission, of his long-term cardiac disease.

The six patients with partial regressions had a median response duration of 3 months (range, 2-4). Their measurable parameters to show these responses were palpable neck nodes (two patients), abnormal liver scan (one), abnormal liver scan and also palpable neck nodes (one), palpable skin metastases (one), and barium esophagram (one).

The median duration of survival was 11 weeks for those patients with measurable disease. It was not different from the 12-week survival of those patients with nonmeasurable, but objectively present, tumor ($P = 0.869$). The median survival of those patients with a favorable pretreatment performance status was 10 weeks. It was 12 weeks for those patients with a poor performance status. Although those patients with increasing disease survived a median of 9 weeks, those with complete or partial response survived a median of 28 weeks ($P = 0.002$). Those patients in the 90-day stable disease category survived a median of 15 weeks. This too was superior to those patients with increasing disease ($P = 0.016$).

Toxicity was significant, but not fatal. Platelet counts $< 50{,}000/mm^3$ were experienced in three of 56 patients. Leukopenia to < 2000 cells/mm^3 was observed in three of the 56 patients. Five patients had anemia resulting in multiple transfusions. In all, 28 patients are described as never having nausea and two are recorded as having nausea without vomiting. Of the 25 patients who had vomiting, in seven patients there were more than six episodes of vomiting after at least one dose. Seventeen of the 56 patients had mild transient elevations of BUN and/or serum creatinine. An additional five patients had greater renal toxicity with transient elevations, with the BUN to 41-60 mg/dl and/or serum creatinine to 2.1-4.0 mg/dl.

Although the overall response rates and survival durations are not as encouraging as those expected from our preliminary report (2), the responders did seem to enjoy a significant survival advantage with a median duration of 28 weeks. The response rates and survival times were impressive in that there were no eligibility restrictions due to performance status. Evidence of the marginal status of the overall patient population is the fact that six of the 45 patients with measurable disease (or seven of all eligible patients) died due to tumor progression, not drug toxicity, within 30 days after entry in the study.

Ravry and Moore (4) reported the effectiveness of 90-mg/m^2 courses of cisplatin. Engstrom et al (5) used 50 mg/m^2 every 3 weeks and reported that five of 25 patients developed partial regressions lasting a median of 11 weeks. The only negative report on single-agent cisplatin is that of Davis et al (6). Only one of the 17 patients treated with 110 mg/m^2 every 4 weeks had a partial response. Perhaps that particular dose regimen was excessively toxic for this particular patient population.

Based on our study and the cumulative experience in the literature, we conclude that, in contrast to its combinations, single-agent cisplatin has only modest effectiveness in the management of squamous cell cancer of the esophagus.

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Randomized Trial of Hexamethylmelamine Versus 5-FU, Doxorubicin, and Cyclophosphamide (FAC) in Advanced Transitional Cell Bladder Carcinoma: A Southwest Oncology Group Study[1,2]

Robert G. Gagliano,[3] Ronald L. Stephens,[4] John J. Costanzi,[5] Noboru Oishi,[6] Walter J. Stuckey,[7] Petre N. Grozea,[8] Jess Frank,[9] and E. David Crawford[10,*]

Chemotherapy for advanced bladder carcinoma with 5-FU produces a 35% response rate (1). Doxorubicin has produced an overall response rate of 28% (2) and cyclophosphamide has produced a response rate of 42% (1) in bladder carcinoma. These single-agent trials used a large variety of doses and schedules, and response criteria and patient selection factors were different from those now accepted for phase II studies. Recent studies indicate lower response rates of 18% for doxorubicin and 31% for cyclophosphamide (3). Combinations of doxorubicin and cyclophosphamide have produced response rates of 17% (4) and 50% (5) in advanced bladder cancer. Combinations of 5-FU and doxorubicin in bladder carcinoma have produced response rates of 35% (6), 50% (7), and 54% (8). Hexamethylmelamine (HEX) has been reported to produce a 40% response rate in bilharzial transitional cell bladder carcinoma (9). Therefore, the Southwest Oncology Group (SWOG) designed a randomized trial comparing HEX to 5-FU, doxorubicin, and cyclophosphamide (FAC) in advanced transitional cell bladder carcinoma. The study was designed to evaluate the regimens in patients with impaired renal function who represent a significant proportion of patients with advanced bladder carcinoma and who would not be eligible for platinum-based chemotherapy regimens.

METHODS AND MATERIALS

Thirty patients with advanced transitional cell bladder carcinoma were entered in the study; 29 were evaluable. Patient characteristics are shown in table 1. All patients had impaired renal function (creatinine clearance 25–75 ml/minute), no prior chemotherapy, and measurable disease. Patients were stratified as good-risk (< 65 years of age) or poor-risk (> 65 years of age or prior radiation therapy) and were randomized to receive either HEX or FAC. Failures were crossed over to receive the other treatment arm. The dose schedules of HEX (10) and FAC (11) have been previously reported. Dose modifications for hematologic toxicity were made according to standard SWOG criteria. Poor-risk patients started chemotherapy with a 25% dose reduction in HEX or FAC.

Complete response was defined as the complete regression of all measurable disease; partial response was defined as a > 50% reduction of the product of the two longest perpendicular diameters of the measurable lesions. Both types of response must last a minimum of 4 weeks. Patients were considered evaluable if they completed one course of chemotherapy and were followed for at least 6 weeks.

RESULTS

No complete response was found on either arm of this study in either the initial or the crossover phase. In the initial phase there was one partial response among 15 patients on the FAC arm and no partial responses among 14 patients on the HEX arm. The one partial response occurred in a patient with pulmonary metastasis; the response duration was 223 days. There were no responses among four patients on the FAC crossover arm and no responses among five patients on the HEX crossover arm. It may be concluded from the results of the initial phase that, with 95% confidence, the probability of complete or partial response is no greater than 32% on the FAC arm and no greater than 23% on the HEX arm.

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[3]Sunrise Hospital, Las Vegas, NV.
[4]University of Kansas Medical Center, Kansas City.
[5]University of Texas at Galveston.
[6]Cancer Center of Hawaii, Honolulu.
[7]Tulane University, New Orleans, LA.
[8]Oklahoma Medical Research Foundation, Oklahoma City.
[9]Southwest Oncology Group Biostatistical Center, Houston, TX.
[10]University of New Mexico, Albuquerque.
Reprint requests to: Southwest Oncology Group (SWOG-7904), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

TABLE 1.—Patient characteristics

Characteristic	No. of patients receiving— HEX	FAC
Age (yrs)		
< 65	3	5
65-69	5	2
70-74	5	3
75+	1	5
Sex		
Male	9	12
Female	5	3
Race		
White	12	11
Black	2	1
Other	0	3
Status		
Good-risk	1	2
Poor-risk	13	13
Prior radiotherapy		
No	11	10
Yes	3	5
Performance status*		
0	1	1
1	4	7
2	6	4
3	4	3
Sites of disease		
Lung	4	5
Node(s)	3	2
Liver	1	0
Skin	1	3
Bladder	5	3
Other	1	2
Toxicity		
Granulocyte count (cells/mm^3)		
> 1500	15	11
< 1500	0	2
< 1000	0	1
< 500	0	1
Thrombocytopenia (cells/mm^3)		
⩾ 100,000	15	13
> 75,000	0	1
> 50,000	0	0
> 25,000	0	1
Gastrointestinal		
None	6	5
Mild	2	4
Moderate	6	5
Severe	1	1

* SWOG criteria.

Median survival duration (measured from start of treatment) was 12 weeks on the FAC arm and 10 weeks on the HEX arm (induction phase). No statistically significant difference in survival duration was found between the two initial treatment arms (2-tailed, log-rank; $P = 0.60$) or between the eight patients < 65 years of age versus the 21 patients ⩾ 65 years of age (2-tailed, log-rank; $P = 0.71$). Although the small number of evaluable patients gives one a reasonable chance of detecting only large differences in survival duration, inspection of survival curves does not suggest any difference regarding either treatment arm or age group.

With the exception of one toxic death (leukopenia and thrombocytopenia) in the FAC arm, no significant difference in type or degree of toxicity was noted between treatment arms.

DISCUSSION

The chemotherapeutic treatment of metastatic bladder cancer remains disappointing. This study underscores this pessimism in that FAC produced only one partial response and HEX produced no responses. There was no difference in the median survival between treatment arms. The low response rate in this study may be partially explained by a preponderance of poor-risk patients (90%) and the stringent criteria for evaluability. We conclude that FAC and HEX are not effective therapy for this disease.

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Combined Iridium 192 Interstitial and External Beam Radiation Therapy for the Treatment of Prostatic Cancer[1]

Bryce S. Clubb* and Jack L. Summers[2]

Twenty-four patients with clinically palpable prostatic adenocarcinoma (stage B or C) were treated with a combination of interstitial radiation therapy and external beam radiation therapy following pelvic lymphadenectomy for accurate staging. Early results indicate a complete clinical response rate at 12 months of 77%, with a 4% complication rate (persisting rectal ulceration). [Cancer Treat Rep 68:1027–1028, 1984]

No one method of delivering radiation therapy for the treatment of early prostatic cancer has achieved universal acceptance.

In January 1982 at this institution a study was begun on a selected group of patients with clinical stage B or C prostatic adenocarcinoma. The study is to be continued over many years to define the advantages and disadvantages of combining a bilateral staging lymphadenectomy, interstitial radiation therapy using a removable afterloading iridium 192 system, and external beam radiation therapy. This initial report concerns the experience gained in the first 16 months of the study and focuses mainly on the technical factors, early responses, and early complications.

MATERIALS AND METHODS

Since January 1982, 24 patients have been entered in the study. All patients have biopsy-proven adenocarcinoma. Presurgical staging investigations, which included whole-body isotope bone scanning and serum acid phosphatase estimations, were all negative. Bipedal lymphangiography was not done as a routine. All patients have had an extra peritoneal laparotomy with exposure of the prostate gland and a formal pelvic lymphadenectomy.

During surgery, the radiation oncologist inserted a total of 18 17-gauge hollow stainless steel needles through the perineum into the pelvic cavity. Twelve tubes completely encircled the prostate and six needles passed into the prostate gland. The needles were kept parallel and 1 cm equidistant by a template which was sutured to the perineal skin. Following surgery and after the patient had been returned to his private hospital room, specially prepared radioactive iridium 192 seeds separated by 1 cm inside small nylon tubing were afterloaded into the metallic needles. The activity of the iridium 192 was 0.33 mCi for the outer 12 nylon tubes and 0.22 mCi for the inner six nylon tubes. This differential method of seed strength distribution produced a more uniform dosimetry throughout the entire tumor volume when compared with using iridium seeds of all the same activity. Computerized dosimetry was done using a program which plots seed positions in relation to x, y, and z planes and produces printouts of isodose lines in the xy, xz, and yz planes. The tumor volume was defined by the isodose line which just encompasses the outer ring of 12 needles, as seen on localization radiographs which were taken in the operating room at the end of the procedure. A tumor dose of 3000 rad was then prescribed to this line and a time was calculated for its delivery. When this time was reached, the iridium 192, template, and tubes were all removed. Two weeks later, all patients began a course of external beam radiation therapy using a 4-MEV linear accelerator. Anterior and posterior portals were used in all patients. The prostate and immediate periprostatic tissues were treated only if the pelvic lymph glands were negative for metastases and a dose of 4000 rad was delivered over a 5-week period. It was felt that the combination of 3000 rad from the interstitial therapy and 4000 rad from the external beam therapy would be sufficient to eradicate macroscopic tumor. In the immediate periprostatic tissue, there was a calculated dose of about 1000 rad from the interstitial therapy. It was felt that the addition of an extra 4000 rad would be sufficient to eradicate possible microscopic tumor in this region. If the

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[2]Departments of Radiation Oncology (*B. S. Clubb*) and Urology (*J. L. Summers*), Akron City Hospital, Northeastern Ohio University College of Medicine.

* *Reprint requests to:* Bryce S. Clubb, MD, Radiation Oncology, 525 E Market St, Akron, OH 44309.

pelvic lymph nodes were positive, then the whole pelvis was included in the anterior and posterior portals and a dose of 5000 rad was delivered over a 6-week period with the prostate area shielded once the tumor dose there had reached 4000 rad.

RESULTS

Surgical staging revealed nine patients with regional pelvic lymph node metastases.

There were no instances of surgical infection or hemorrhage associated with the interstitial radiation therapy. Of nine patients followed for > 12 months, seven (77%) have had a complete clinical response, meaning that the gland has returned to normal size and consistency. Of the seven complete responses, five were stage B2 tumors and two were stage C tumors. Two patients (22%) had a partial response, meaning that their prostate glands were reduced in size but had not yet returned to normal size or consistency. One of these patients was stage B2 and the other was stage C. Fifteen patients in the series have yet to reach 1 year and cannot yet be adequately evaluated for full response. Four patients (8%) developed proctitis with rectal ulceration. This was reversed in two patients using dietary regulation and hydrocortisone enemas. Two patients have a persistence of rectal ulceration and require continuing medication. Diverting colostomy has not yet been required.

DISCUSSION

The optimum use of radiation as potentially curative treatment for prostatic carcinoma has remained very controversial since its introduction in 1910 when Pashkis and Tittinger (1) first introduced radium-containing catheters into the prostatic urethra. The use of our removable afterloading system is felt to offer a number of advantages:

(*a*) The use of a perineal template with holes accurately placed in a circular fashion helps to keep the needles equidistant and parallel while they are being inserted into the prostate gland. The radiation oncologist is able to reposition needles that do not appear in correct position before the iridium seeds are loaded.

(*b*) Afterloading of the radioisotope eliminates the risk of radiation exposure to surgical personnel in the operating room.

(*c*) By varying the radiation activity of the iridium 192 seeds in the metallic needles and using computerized dosimetry, an accurate dose of radiation with optimum dose distribution can be delivered to the macroscopic primary tumor. Because of the proximity of the rectum, bladder neck, and urethra to the implant volume, the dose from the iridium was limited to 3000 rad.

The addition of external beam radiation therapy increases the total dose in the macroscopic tumor toward cancericidal levels. The finding of metastases in the regional pelvic lymph nodes unquestionably indicates a poorer prognosis. There is some evidence that external beam radiation therapy may offer some survival benefit to patients with pelvic lymph node metastases (2). However, surgical staging helps to avoid large external beam radiation therapy portals for those patients with negative lymph nodes and may be helpful in minimizing radiation complications which are more likely to occur if radiation therapy is given to the pelvis following a formal pelvic lymphadenectomy.

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Dyspnea Following Vinblastine or Vindesine Administration in Patients Receiving Mitomycin Plus Vinca Alkaloid Combination Therapy[1,2]

Mark G. Kris,[3,*] Dagmara Pablo,[4] Richard J. Gralla,[3] M. Thomasine Burke,[3] Judith Prestifilippo,[4] and Diana Lewin[4,5]

Pulmonary toxicity is not commonly associated with the vinca alkaloids vinblastine and vindesine. Three patients receiving the combination of mitomycin plus vindesine or vinblastine developed acute dyspnea 1–5 hours following vinca alkaloid administration. Two of the three patients continued to receive therapy and both experienced symptoms with each subsequent vinblastine or vindesine injection. No other cause for the dyspnea could be detected. We conclude that, as in the three previously reported cases, shortness of breath can occur after the injection of vinblastine or vindesine in patients receiving mitomycin plus vinca alkaloid combination therapy. [Cancer Treat Rep 68:1029–1031, 1984]

Pulmonary toxicity caused by cytotoxic agents has been well-documented (1). Large series of patients in which the vinca alkaloids vincristine (2), vinblastine (3), and vindesine (4) were used as single agents do not mention lung toxicity as an adverse effect. In 1978, Israel and Olsen (5) described a syndrome of dyspnea and pulmonary infiltrates following vinblastine administration in a patient with breast cancer who received combination chemotherapy with vinblastine and mitomycin. Konits et al (6) have presented similar cases. Here we report three additional patients receiving a vinca alkaloid and mitomycin who developed acute dyspnea related to the injection of vinblastine or vindesine.

CASE REPORTS

Patient 1

A 63-year-old woman was found to have adenocarcinoma of the lung metastatic to the humerus. She received 32 courses of etoposide over 25 months. After relapse of tumor, the patient received three courses (159 mg) of deoxydoxorubicin. After progression of the adenocarcinoma, she was given a total of 26 mg/m^2 of vindesine and 30 mg/m^2 of mitomycin over a 4-month period without complication. A partial remission of her tumor occurred. Five hours following the administration of the tenth vindesine dose and the fourth mitomycin dose, she acutely developed dyspnea that persisted for 3 hours. No new abnormalities on physical examination, chest x-ray, or ECG were noted. A ventilation-perfusion lung scan did not document pulmonary emboli. No eosinophilia was observed. No specific therapy was given.

Two weeks later, the patient received vindesine alone and after 1 hour, shortness of breath developed suddenly, lasted 3 hours, and was relieved by nasal oxygen. Her physical examination was unchanged. Two weeks thereafter, she received vindesine alone, immediately preceded by 200 mg of methylprednisolone. She again experienced severe dyspnea 3 hours later. The physical examination revealed a regular tachycardia, tachypnea, and wheezes over both lung fields. Increased interstitial markings were noted bilaterally on chest x-ray. The ECG showed atrial bigeminy. Arterial blood gases (nasal oxygen at 5 L/minute) were pH 7.40, pCO_2 30 mm Hg, and pO_2 70 mm Hg. A ventilation-perfusion lung scan was unchanged. The patient improved over a 6-hour period with nasal oxygen, isoetharine, and aminophylline. Spirometry revealed moderate obstructive lung disease. The ECG returned to

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[3]Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY.
[4]St. Michael's Medical Center, Newark, NJ.
[5]We thank Jean Elbaum for preparation of the manuscript.
*Reprint requests to: Mark G. Kris, MD, Developmental Chemotherapy Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

baseline. Creatine phosphokinase, LDH, and SGOT were normal. A chest x-ray 2 weeks later showed improved but persistent lower lobe infiltrates. Vindesine and mitomycin were discontinued and symptoms have not recurred.

Patient 2

A 53-year-old woman underwent a mastectomy for stage III breast cancer and received cyclophosphamide, methotrexate, and 5-FU for 1 year, then 6 months of melphalan. Three years later, lung nodules and bony metastases appeared. She then received cyclophosphamide, doxorubicin, and 5-FU for 9 months. She returned 8 months later after a subcutaneous mass developed. She was then given a total dose of 50 mg/m^2 of mitomycin and 60 mg/m^2 of vinblastine over 7 months with objective antitumor response and without complications.

Three hours after receiving her sixth mitomycin and 11th vinblastine dose, the patient returned to the hospital complaining of severe shortness of breath, dry cough, and pleuritic chest pain. Physical examination revealed a regular tachycardia and bilateral wheezes. Laboratory data included: an unchanged chest x-ray with pulmonary nodules; no eosinophilia; arterial blood gases (room air)—pH 7.32, pCO_2 35 mm Hg, and pO_2 85 mm Hg; spirometry—severe obstructive lung disease; cardiac scan—good left ventricular function with ejection fraction of 53%; ventilation-perfusion lung scan—findings consistent with airway disease, no evidence of pulmonary embolism; and ECG—sinus tachycardia, nonspecific ST-T changes. Isoetharine, methylprednisolone, and erythromycin were administered and dyspnea improved. Twenty-four-hour ECG monitoring revealed sinus tachycardia and rare ventricular premature contractions.

The patient did well except for dyspnea on exertion. Two weeks later, she received 6 mg/m^2 of vinblastine. Two hours afterward, she developed shortness of breath and wheezing. Physical examination revealed tachypnea, tachycardia, diaphoresis, neck vein distension, and diffuse bilateral wheezes. Arterial blood gases (3 L of nasal oxygen) were pH 7.03, pCO_2 83 mm Hg, and pO_2 81 mm Hg. The chest x-ray demonstrated increased interstitial markings. She was treated with furosemide, epinephrine, aminophylline, and diphenhydramine and required endotracheal intubation. Two grams of methylprednisolone were given every 6 hours for four doses. She improved over the next 24 hours and was extubated. A ventilation-perfusion lung scan and ECG were unchanged from the studies done 2 weeks previously. Serial chest x-rays showed progressive improvement in the interstitial lesions. Mitomycin and vinblastine were discontinued. The patient continues to have dyspnea.

Patient 3

A 54-year-old woman was found to have adenocarcinoma of the lung presenting as Pancoast's syndrome. After an initial response with radiotherapy, bony lesions worsened and she was given 21 mg/m^2 of vindesine and 30 mg/m^2 of mitomycin (three doses) over a 3-month period with improvement in symptoms and no adverse effects. One hour after receiving her eighth vindesine injection, she acutely developed dyspnea and cough. Physical examination revealed a regular tachycardia, tachypnea, and bilateral wheezes. Laboratory data included: arterial blood gases (room air)—pH 7.46, pCO_2 29 mm Hg, and pO_2 36 mm Hg; chest x-ray—blunted costophrenic angles, increased interstitial markings; two-dimensional echocardiogram—normal; pulmonary angiogram—normal; ECG—normal; creatine phosphokinase, LDH, and SGOT—normal; no eosinophilia. The patient received bronchodilators, corticosteroids, and oxygen and gradually improved over 7 days. Mitomycin and vindesine were discontinued and symptoms have not recurred.

DISCUSSION

A syndrome of dyspnea and pulmonary infiltrates following vinblastine administration in patients receiving mitomycin and vinblastine combination chemotherapy has been described in three women with breast cancer. The three patients presented in this report received mitomycin with a vinca alkaloid and developed acute dyspnea 1–5 hours following the injection of vindesine or vinblastine. Lung infiltrates on chest x-ray were not consistently present. No patient had experienced a similar prior illness or had a history of symptomatic cardiopulmonary disease. Because of significant antitumor effect, Patients 1 and 2 were given additional vinca alkaloid injections and again developed symptoms. Because of that experience, the drugs were discontinued after the first episode in Patient 3. Based on the temporal relationship to the vinca alkaloid injections, the occurrence with each rechallenge, and with further investigation failing to reveal another cause for the dyspnea, we attribute the acute shortness of breath to the administration of the vinca alkaloids in these patients who previously or simultaneously were treated with mitomycin.

The pathophysiology of this syndrome is not known. Since there are no reports of therapeutic doses of the vinca alkaloids alone causing lung toxicity, the pulmonary damage may be caused by either the combination of vinca alkaloid and mitomycin or by potentiation of mitomycin-induced or other prior cytotoxic drug-induced lung toxicity by vinblastine or vindesine. Progressive dyspnea and nonproductive cough, hypoxia, diffusion abnormalities, and interstitial pneumonitis

have all been previously described in patients receiving mitomycin (1). Whether pretreatment with antihistamines or corticosteroids given in a dose and schedule other than that used in Patient 1 could prevent or attenuate these symptoms is also not known. The possibility of persistent or progressive pulmonary toxicity, despite discontinuation of medication, cannot be excluded.

The true incidence of this syndrome is not known. Patients 1 and 3 were part of a 90-patient trial investigating vindesine and mitomycin in non-small cell lung cancer in which a total of three patients developed acute dyspnea, giving an incidence of 3% for this particular study (7). With a total of six reported cases and the increased usage of the vinca alkaloids and mitomycin in breast cancer (8) and non-small cell lung cancer (9), treating physicians should be aware of this possible complication.

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Nonaggressive Combination Chemotherapy for Resistant Immunocytoma[1]

David Bareford,* Derwood Pamphilon, and David Barnard[2]

Two patients with advanced immunocytomas who had become resistant to conventional chemotherapy with developing pancytopenia were given cyclical courses of bleomycin, doxorubicin, vindesine, and prednisolone. Both patients showed an excellent response with recovery of peripheral blood cell counts, marked reduction in marrow involvement, and an increased sense of well-being. [Cancer Treat Rep 68:1033–1034, 1984]

Treatment of immunocytomas such as Waldenström's macroglobulinemia with chlorambucil shows a 40%–60% response rate (1,2). Once the tumor becomes resistant to alkylating agents, the prognosis is poor (3). Treatment with more aggressive agents at this stage is often made difficult by developing pancytopenia resulting from previous alkylating agent therapy and increasing bone marrow involvement. A four-drug chemotherapy regimen was devised in which some activity against immunocytomas was expected, while accompanying myelosuppression was limited.

CASE REPORTS

Patient 1

A 66-year-old patient presented in March 1980 with a 3-week history of lethargy, headache, shortness of breath, and bilateral claudication. Upon examination, he had signs of the hyperviscosity syndrome and a 5-cm hepatomegaly. Tests revealed a Hgb level of 6.8 g/dl, a wbc count of 4.8×10^9/L (38% neutrophils, 50% lymphocytes, and 8% plasmacytoid lymphocytes), and a platelet count of 168×10^9/L. Monoclonal IgM kappa was 77 g/dl, plasma viscosity was 7.1 cp, and there was bone marrow infiltration by lymphoplasmacytoid cells. A diagnosis of immunocytoma of lymphoplasmacytoid type was made. After initial steroids and plasma exchange, the patient was started on chlorambucil (4 mg/day), which was discontinued after 3 weeks due to marked pancytopenia. A repeat marrow still showed heavy infiltration and the IgM level was unchanged. Therapy was changed to cyclical cyclophosphamide, vincristine, and prednisolone, with good response. The paraprotein fell to 27 g/L, but the vincristine had to be withdrawn due to severe neuropathy. A marrow at this point showed improvement.

The patient continued on cyclophosphamide (900 mg iv) and prednisolone (40 mg/day for 7 days) every month. He remained in good health for 2 years, although there was a gradual increase in IgM level to 40 g/L. By September 1982, the patient was requiring transfusion support, becoming increasingly pancytopenic, and beginning to feel weak with frequent headaches. The marrow aspirate and trephine showed heavy involvement by lymphoplasmacytoid cells.

The patient started on cyclical chemotherapy with bleomycin (15 mg iv) on Days 1 and 2, doxorubicin (25 mg/m^2 iv) on Days 1 and 2, vindesine (5 mg) on Day 1, and prednisolone (60 mg/day) for 7 days every 4 weeks. The peripheral blood cell count improved and his sense of well-being increased dramatically. The IgM level fell to 16 g/L and the bone marrow trephine showed improvement with residual small foci of plasmacytoid cells in normal hemopoiesis. After six courses, therapy was stopped. Toxicity was limited to alopecia. Nine months later, the patient remains well.

Patient 2

A 67-year-old patient presented in July 1981 with a 3-month history of pallor, lack of energy, and weight loss. Upon examination, there was a 10-cm splenomegaly; there was no hepatomegaly or lymphadenopathy. The patient had a Hgb level of 8.7 g/dl, a wbc count of 15×10^9/L (78% neutrophils and 5% plasmacytoid lymphocytes), and a platelet count of 220×10^9/L. Monoclonal IgA kappa was 22 g/L. Bone marrow trephine showed marked follicular infiltration by pleomorphic lympho-

[1]Received Aug 2, 1983; revised Jan 26, 1984; accepted Feb 7, 1984.

[2]Department of Haematology, St. James' University Hospital, Leeds, England.

**Reprint requests to:* Dr. David Bareford, Department of Haematology, The Queen Elizabeth Hospital, Queen Elizabeth Medical Centre, Edgbaston, Birmingham B15 2TH, England.

cytes, plasmacytoid lymphocytes, and plasma cells. A diagnosis of immunocytoma of polymorphic type was made. The patient was started on chlorambucil (6 mg/day) and after 3 months, the spleen had become impalpable and the wbc count was normal, although the IgA level remained static. Chlorambucil was continued at a reduced dose until October 1982 when the spleen rapidly expanded to 10 cm with a rising peripheral lymphocyte count. A trial of cyclophosphamide and then an increase in chlorambucil to 10 mg/day had no effect.

At this stage, the patient was transfusion-dependent with thrombocytopenia. The IgA was 16 g/L. The patient started on bleomycin, doxorubicin, vindesine, and prednisolone, as with Patient 1. The first course included 10 mg of vindesine, which was found to be too myelosuppressive. There was a rapid shrinkage in the spleen size to 1 cm and an increased sense of well-being. A bone marrow after one course showed a hypocellular marrow with a few small residual follicles of pleomorphic lymphoid cells. The patient continues through six courses, although delays have occurred due to viral infections. Toxic effects have been limited to moderate alopecia and an episode of mucositis after the fourth course.

DISCUSSION

Both patients have a lymphoproliferative disorder represented in the Keil classification by the term immunocytoma (4). These are non-Hodgkin's lymphomas with plasmacytic features and include classical Waldenström's macroglobulinemia, lymphomas secreting other monoclonal proteins, and some lymphomas unassociated with paraprotein secretion. Patients with macroglobulinemia have a reported average survival of 49 months for responding groups and 24 months for the nonresponders (5). Patients with IgA- and IgG-secreting immunocytomas were originally reported as having a fairly benign course similar to Waldenström's disease (2), but a more recent report of 12 patients suggests that the course of the disease may be more aggressive (6). It is certain that once resistance develops to alkylating agents, the prognosis is poor (3).

A combination of doxorubicin, bleomycin, and prednisone has been shown to be active in other non-Hodgkin's lymphomas resistant to cyclophosphamide, vincristine, and prednisolone (7), and the M-2 protocol (melphalan, prednisolone, cyclophosphamide, vincristine, and carmustine) has been tried with some success in macroglobulinemia (8). As a single agent, doxorubicin has been shown to have activity in two patients with macroglobulinemia resistant to alkylating agents (9). One study (10) found vindesine to have a 55% response rate in alkylating agent-resistant myeloma, but a recent report found no response to vindesine unless prednisone was added (11). Vindesine appears to have a low level of activity as a single agent in non-Hodgkin's lymphoma (12). Bleomycin is known to be an active chemotherapeutic agent in non-Hodgkin's lymphoma and has the advantage of being relatively nonmyelosuppressive (13).

This combination of bleomycin, doxorubicin, vindesine, and prednisolone was chosen for the known activities of the individual drugs and for the relative nonmyelosuppressive properties of three of the agents. Because immunocytomas are relatively rare, the effect of each of the drugs used alone in this condition is unknown and the four-drug combination may contain an ineffective agent. Both patients showed a good response with negligible toxic effects.

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Concomitant Razoxane and Doxorubicin Treatment on a Weekly Schedule[1,2]

Rowan T. Chlebowski,* Reginald P. Pugh, Francis Bull, Joseph McCracken, Andrew Glass, and Joseph R. Bateman[3]

The use of doxorubicin given on a weekly schedule has been associated with a reduced incidence of drug-induced congestive heart failure (1,2). Razoxane (ICRF-159) is a member of the dioxopiperazine family. In a variety of systems, evidence has been reported which suggests that these agents protect against the development of anthracycline cardiotoxicity (3-5). As a result, a combination regimen combining weekly concomitant ICRF-159 with doxorubicin might further reduce the risk of drug-induced cardiotoxicity. In addition, synergistic cytotoxicity between anthracyclines and ICRF-159 has been observed in vitro (6). Therefore, to determine the acute toxic effects of concomitant doxorubicin and ICRF-159 use, we prospectively evaluated a weekly schedule of doxorubicin given iv in combination with ICRF-159 given orally at four dosage levels.

METHODS

All patients had histologic diagnosis of malignancy and measurable, unresectable disease. All patients with lung cancer had metastatic rather than locally advanced disease. The median age of study patients was 61 years (range, 19-78) and the median Karnofsky performance status was 75%. Pretreatment evaluations included cbc, BUN, liver function tests, ECG, and chest roentgenograms. Ongoing studies to monitor cardiac function were not performed. Laboratory values required for entry included total wbc count $> 4000/mm^3$; platelet count $> 150{,}000/mm^3$; hematocrit $> 28\%$; bilirubin < 5 mg/dl; and creatinine clearance > 50 mg/minute. A complete response was defined as the disappearance of all measurable evidence of tumor. Partial response was defined as a $> 50\%$ reduction in the sum of the product of the diameters of all measurable lesions, provided no lesions increased in size and no new lesions developed. All responses were of at least 1 month's duration. Patients receiving one dose of doxorubicin and ICRF-159 were evaluable for toxicity and response.

All patients received ICRF-159 given orally within 30 minutes prior to rapid iv administration of doxorubicin. Therapy was repeated on a weekly basis. Dosage was not adjusted for bilirubin level. Four dosage levels were given, with subsequent patient groups receiving higher dosage levels of both agents. Dosage was not escalated in any individual patient. Level I patients received 1.5 g/m^2 of ICRF-159 and 15 mg/m^2 of doxorubicin; level II patients received 2.0 g/m^2 of ICRF-159 and 20 mg/m^2 of doxorubicin; level III patients received 2.5 g/m^2 of ICRF-159 and 25 mg/m^2 of doxorubicin; and level IV patients received 3.0 g/m^2 of ICRF-159 and 30 mg/m^2 of doxorubicin. A group of patients was studied at each dosage level and evaluated before additional patients were entered at subsequent dosage levels. Individual patient dosage was adjusted on the basis of hematologic toxicity. Patients with persistent (> 2 weeks) leukopenia (between 2900 and 2000 cells/mm^3) had the doxorubicin dose reduced by 5 mg/m^2 and the ICRF-159 dose reduced by 500 mg/m^2.

RESULTS AND DISCUSSION

A total of 48 previously untreated patients were entered in the study, with all patients evaluable for toxicity and response. Only four patients had bilirubin levels > 1.5 mg/dl. Most common patient diagnoses included lung cancer (20 patients with non-small cell and two with small cell), sarcoma (four with osteogenic and ten with soft tissue), renal cell carcinoma (four), bladder carcinoma (three), and adenocarcinoma of unknown primary (five). Eleven patients received treatment level I, 15 received treatment level II, 16 received treatment level III, and six received treatment level IV.

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* *Reprint requests to:* Rowan T. Chlebowski, MD, Division of Medical Oncology, Department of Medicine, UCLA School of Medicine, Harbor-UCLA Medical Center, 1000 W Carson St, Torrance, CA 90509.

TABLE 1.—Toxic effects of doxorubicin and ICRF-159 on a weekly schedule by dosage level

Toxic effect	% of patients with toxic effect by dosage level*			
	I (n = 11)	II (n = 15)	III (n = 16)	IV (n = 6)
Hematologic				
Leukopenia (cells/mm^3)				
2999–2000	36	33	44	50
1999–1000	18	13	13	33
< 1000	0	7	13	17
Thrombocytopenia (cells/mm^3)				
99,999–50,000	0	0	9	33
< 50,000	9	7	6	17
Gastrointestinal				
Nausea and vomiting				
Moderate	27	33	25	33
Severe	0	0	13	17
Stomatitis	9	7	6	16

*Level I = doxorubicin, 15 mg/m^2 and ICRF-159, 1.5 g/m^2; level II = doxorubicin, 20 mg/m^2 and ICRF-159, 2.0 g/m^2; level III = doxorubicin, 25 mg/m^2 and ICRF-159, 2.5 g/m^2; and level IV = doxorubicin, 30 mg/m^2 and ICRF-159, 3.0 g/m^2.

Toxic effects resulting from weekly combination ICRF-159 and doxorubicin therapy by dosage level can be seen in table 1. The major dose-limiting toxic effect was hematologic. Analysis of the six patients treated at dosage level IV indicated a rather uniform toxicity experience. Leukopenia < 3000 cells/mm^3 occurred on about Day 14 after two weekly doses of doxorubicin and ICRF-159 were completed. If the third weekly dose was administered, the leukopenia progressed to < 2000 cells/mm^3 on Day 21. This pattern was also seen in some of the patients on level III. Twenty-six percent of the patients treated with level III or level IV had a granulocyte count nadir < 500/mm^3. Alopecia was documented in 39% of the patients. Gastrointestinal toxicity was not severe and was most frequently seen as nausea and vomiting (35% of the patients). Stomatitis, however, was uncommon and was seen in only 8% of the patients. No identifiable renal, pulmonary, or hepatic toxicity was seen. No patient developed clinical evidence of doxorubicin-induced congestive heart failure in this trial; however, few patients experienced prolonged survival, and only seven patients received > 450 mg/m^2 of doxorubicin. The cumulative median doxorubicin dose was 163 mg/m^2, with a mean dose of 267 ± 43 (mean ± SEM).

Evaluation of therapeutic efficacy was not a major goal of the toxicity-seeking study design used in this trial. Nonetheless, partial objective responses were seen in patients with lung cancer (23% response rate), sarcoma (38% objective response rate; 14% complete response rate), renal cell carcinoma (25% response rate), and bladder carcinoma (33% response rate), but not in adenocarcinoma of unknown primary. Only 9% of patients receiving treatment level I (doxorubicin, 15 mg/m^2) responded compared to a somewhat higher response frequency of 30% in patients receiving treatment levels II, III, or IV, but this difference did not achieve statistical significance.

Poor oral absorption has been documented for ICRF-159, with absorption decreasing as bolus dosage increased (7). These pharmacokinetics are consistent with the phase I results in which similar myelosuppression was seen at single-agent ICRF-159 oral, bolus doses of between 1.0 and 7.5 g/m^2 (8). Therefore, the dose-limiting hematologic toxicity seen at treatment level IV in the present study was most likely due to the increasing doxorubicin rather than ICRF-159 dose. These results suggest that future combination trials with these agents should not include ICRF-159 doses > 1 g/m^2.

In summary, ICRF-159 can be safely given concomitantly with doxorubicin on a weekly schedule of administration if appropriate adjustments for enhanced hematologic toxicity are made. The objective responses seen with the ICRF-159 and doxorubicin combination were comparable to those reported previously for single-agent weekly doxorubicin in the diseases treated (2). Based on preclinical evidence, a weekly doxorubicin and ICRF-159 combination might result in a reduced degree of cardiac injury (3–5). Our results suggest a dose of between 20 and 25 mg/m^2 of doxorubicin with 1.0 g/m^2 of ICRF-159 for further studies designed to prospectively evaluate the potential of ICRF-159 to influence doxorubicin-induced cardiotoxicity.

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Sequential Continuous Infusion With Doxorubicin and Vinblastine: An Effective Chemotherapy Combination for Patients With Advanced Breast Cancer Previously Treated With Cyclophosphamide, Methotrexate, 5-FU, Vincristine, and Prednisone[1]

Nizar Tannir,* Hwee-Yong Yap, Gabriel H. Hortobagyi, Verena Hug, Aman U. Buzdar, and George R. Blumenschein[2,3]

Forty-two patients with metastatic breast carcinoma previously treated with a combination of cyclophosphamide, methotrexate, and 5-FU, with or without vincristine or prednisone, were treated at relapse with a sequential combination of doxorubicin and vinblastine given by continuous infusion. Two patients achieved complete remission and 16 achieved partial remission for a total response rate of 43%. The median time to progression for responders and patients with stable disease was 10 and 4 months, respectively. Neutropenia was the major toxic effect but did not lead to any fatality. [Cancer Treat Rep 68:1039–1041, 1984]

Although the use of doxorubicin in combination with other effective agents has been shown to achieve a higher response rate and a longer response duration in comparison with the most effective non-doxorubicin combinations (1,2), there are still a large number of patients who are treated with cyclophosphamide, methotrexate, and 5-FU (CMF) as adjuvant chemotherapy for operable breast cancer or as front-line treatment of metastatic disease. In previous studies of patients whose treatment with CMF eventually failed, single agents or combinations of drugs have usually yielded 20%–40% response rates, and responses have lasted from 4 to 6 months (1). The response rate to doxorubicin as a single agent in previously treated patients with metastatic breast cancer is 15%–28% (3). As a single agent, vinblastine has a response rate in the range of 20% when given by iv bolus (4). We have previously suggested an improved therapeutic index when doxorubicin or vinblastine was given as a continuous infusion in patients with metastatic breast cancer (5,6). In this study, we explored the sequential combination of doxorubicin and vinblastine given by continuous infusion for patients with metastatic breast cancer who have failed prior CMF therapy.

MATERIALS AND METHODS

Forty-two consecutive patients with metastatic breast cancer previously treated with CMF were entered in this study. Patients were considered eligible if they met the following criteria: (*a*) biopsy-proven adenocarcinoma of the breast; (*b*) measurable progressive disease; (*c*) no prior exposure to doxorubicin or other anthracyclines; (*d*) no prior chemotherapy for at least 3 weeks; (*e*) no prior hormonal therapy for at least 4 weeks; and (*f*) signed informed consent. Patients with evidence of congestive heart failure were not eligible for this study. Doxorubicin was given at the starting dose of 25 mg/m^2 as continuous iv infusion over 24 hours × 2 days, followed by vinblastine at the starting dose of 1.4 mg/m^2 as continuous iv infusion over 24 hours × 4 days. The cycle was repeated every 3 weeks after recovery from hematologic and other toxic effects. The doses of doxorubicin and vinblastine were later modified according to blood counts (table 1). Prior to treatment, the patients were evaluated by physical examination, cbc, platelet count, biochemical screen (SMA-12) and CEA, chest radiograph, liver scan and/or ultrasound, bone scan and skeletal survey, bone mar-

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[2]Division of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

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**Reprint requests to:* Dr. Nizar Tannir, Box 10, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Dose modification of doxorubicin and vinblastine according to blood counts

Lowest neutrophil count (/mm^3)		Lowest platelet count (/mm^3)	Dose modification
> 1500	and/or	> 100,000	20% increase (both drugs)
> 750 but < 1500	and/or	> 75,000 but < 100,000	No change
< 750	and/or	< 75,000	20% decrease (both drugs)

row aspirate and biopsy, brain scan, ECG, and cardiac nuclear scan. Weekly cbc, monthly SMA-12 and CEA, and radiologic or nuclear scans (every 2 months) were done to assess efficacy and toxicity of the regimen. Responding patients were considered for endomyocardial biopsy at the completion of 450 mg/m^2 of doxorubicin. Doxorubicin was stopped if either grade 2 myocardial damage was documented by endomyocardial biopsy or if the patient refused biopsy when the cumulative dose of doxorubicin (450 mg/m^2) was reached. In responding patients who have shown no evidence of doxorubicin-induced cardiac toxicity at 450 mg/m^2, therapy with doxorubicin was continued, and subsequent endomyocardial biopsies were carried out at 250-mg/m^2 increments. Standard criteria for response were utilized.

RESULTS

The pretreatment patient characteristics are presented in table 2. There were two complete and 16 partial responses (43% total response rate; the 95% confidence interval ranges from 27% to 59%). The time interval from the date of discontinuation of CMF to the date of initiation of doxorubicin and vinblastine was a statistically significant factor that predicted response. The median of this interval was 9 months (range, 0–36) for responders and 3 months (range, 0–34) for nonresponders (P = 0.01). The median time to progression for responders and patients with stable disease was 10 months (range, 4–19+) and 4 months (range, 2–9), respectively. Toxic effects are presented in table 2. Thrombocytopenia was mild to moderate in severity; neutropenia was severe and led to four episodes of septicemia and one episode of pneumonia and bacteremia. All of the patients who developed an infection or fever associated with neutropenia were successfully treated. Four patients received a total cumulative dose of doxorubicin of 850, 800, 690, and 650 mg/m^2 without cardiac toxicity.

DISCUSSION

Chemotherapy combinations incorporating doxorubicin with other effective cytotoxic agents have consti-

TABLE 2.—Pretreatment patient characteristics and treatment results

Characteristic	% of patients
Median age in yrs (range): 50 (30–71)	
Performance status*	
0	18
1	51
2	21
3	10
Dominant site of metastasis	
Viscera	54
Bone	31
Soft tissue	15
No. of metastatic sites	
1	44
2	28
3	28
Prior hormonal therapy	44
Prior radiation therapy	60
Prior CMF chemotherapy	
Adjuvant	72
Front-line	28
Response	
Complete	5
Partial	38
Toxic effects	
Alopecia	100
Nausea and vomiting (mild)	70
Constipation	11
Fever associated with vinblastine	11
Mucositis (mild)	8
Paresthesias	8
Myalgias	5
Fever associated with neutropenia	16
Septicemia	10
Pneumonia	5
Urinary tract infection	3
Bleeding	3
Platelet count (/mm^3)	
< 20,000	7
< 50,000	7
< 100,000	24
Neutrophil count (/mm^3)	
< 100	18
< 500	79
< 1000	100

* International Union Against Cancer criteria.

tuted the most widely used second-line regimens for patients with recurrent metastatic breast cancer previously exposed to CMF. With the combination of doxorubicin and vincristine, the response rate was 25% (7). Combinations with lomustine or semustine did not result in a higher response rate when compared with the administration of doxorubicin alone (8,9). When mitomycin was administered with doxorubicin, the response rate was 40%, but significant organ toxicity was a major problem (10). In this study, our results with doxorubicin and vinblastine are comparable to those reported with other second-line regimens (11–13). The results also suggest that doxorubicin and vinblastine may be the critical agents in the VATH (vinblastine, doxorubicin, thiotepa, and fluoxymesterone) combination, which is regarded as the most effective second-line combination reported so far (12). We conclude that for patients who have failed CMF chemotherapy, the regimen of doxorubicin and vinblastine is worthy of further study.

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QT Interval Prolongation and Cardiac Dysrhythmia in a Patient Receiving Amsacrine[1,2]

Cindy L. Schwartz,* Karen S. Bender, Philip J. Burke, Jean S. Kan, and Curt I. Civin[3]

Amsacrine (AMSA), an acridine derivative, has been shown to have antineoplastic activity, especially in leukemia (1-3). Like the anthracyclines, AMSA probably exerts its effect by intercalating with DNA (4). Acute cardiac dysrhythmias, during and following AMSA administration, have been reported (5-7), most in association with hypokalemia. We describe a young woman who, while normokalemic, developed an AMSA-related prolongation of the corrected QT (QTc) interval, followed by ventricular tachycardia. We speculate that QTc prolongation may precede and predispose to AMSA-related dysrhythmia.

CASE REPORT

In October 1981, a 15¼-year-old female developed pre-B-cell acute lymphocytic leukemia. Remission was induced, consolidated, and maintained with a regimen that included prednisone, vincristine, daunorubicin, L-asparaginase, cytarabine (Ara-C), methotrexate, 6-mercaptopurine, and triple intrathecal medications (methotrexate, Ara-C, and hydrocortisone). Her leukemia relapsed in June 1982 and reinduction was attempted with high-dose Ara-C (8) and daunorubicin. By August 1982, the patient had received a cumulative total daunorubicin dose of 240 mg/m^2 and her ejection cardiac fraction by nuclear imaging was 54% (normal, 50%-80%). A second course of high-dose Ara-C and daunorubicin was administered, increasing her cumulative daunorubicin dose to 300 mg/m^2, without producing clinical evidence of cardiac failure. Remission was not achieved and treatment was begun (with an informed consent) with a regimen (CAMP:JHOC 8303) of cyclophosphamide (3 g/m^2) on Day 1, AMSA (120 mg/m^2) on Days 8-12, and etoposide (250 mg/m^2) on Days 15 and 16. Her "pre-AMSA" ECG on Day 7 was normal, with a QTc interval of 0.42 second (table 1), but her "pre-AMSA" ejection fraction on Day 8 (immediately before AMSA, 7 days after cyclophosphamide, and 6½ weeks after the last daunorubicin dose) had declined to 41%. The patient had no signs of cardiac failure. The first dose of AMSA (240 mg in 250 ml of a 5% dextrose solution over 2 hours) was administered without complication. On Dav 9, prior to her second AMSA infusion, an ECG was normal, except for a prolonged QTc interval of 0.50 second. On Day 10, prior to receiving AMSA, her QTc interval was 0.47 second. Serum electrolytes obtained that morning were normal (potassium, 3.8 mEq/L; calcium, 9.2 mEq/L; and magnesium, 1.5 mEq/L). During the third AMSA infusion (Day 10), the patient had nausea and chest discomfort, then developed ventricular tachycardia and became unresponsive. AMSA was immediately discontinued; normal sinus rhythm returned spontaneously, and the patient awoke. After this event, QTc interval was 0.52 second, serum potassium was 3.3 mEq/L, and other electrolytes were within normal limits. No further AMSA was administered. Lidocaine was begun prophylactically and continued for 3 days. During Days 8-10, other medications received were megestrol acetate, allopurinol, and small doses of heparin (for maintaining iv catheter patency). Etoposide was given earlier than scheduled (Days 11 and 12). The QTc interval gradually returned to normal during the next 2 weeks. No further dysrhythmias or cardiac dysfunctions were noted clinically, by ECG, or by chest x-ray prior to the patient's death on Day 60 with recurrent leukemia. Other cardiac studies were not done on this terminal patient and no autopsy was performed.

DISCUSSION

Prolongation of the QTc interval predisposes to cardiac dysrhythmias and sudden death (10). In this patient, QTc interval prolongation preceded the development of a life-threatening AMSA-related dysrhythmia and may have represented early evidence of AMSA-induced cardiotoxicity. AMSA, like the anthracyclines, is toxic in vitro

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[3]Division of Pediatric Oncology (*C. L. Schwartz* and *C. I. Civin*), Adult Leukemia Service (*P. J. Burke*), Department of Pediatrics (*C. L. Schwartz, K. S. Bender, J. S. Kan*, and *C. I. Civin*), and Division of Pediatric Cardiology (*J. S. Kan*), The Johns Hopkins Oncology Center, Baltimore, MD.

* *Reprint requests to:* Cindy L. Schwartz, MD, The Johns Hopkins Hospital, Oncology Center 3-121, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 1.—QT interval in relation to heart rate and serum potassium *

Date	Heart rate by ECG (beats/min)	QT interval (sec)	QTc interval(s) (sec)	Potassium (mEq/L)	Comments
Nov 16, 1981	95	0.32	0.42	ND	Partial remission
Dec 29, 1981	66	0.40	0.42	4.2	Complete remission
Mar 17, 1982	60	0.36	0.36	4.2	Complete remission
July 2, 1982	66	0.40	0.42	3.9	Relapse
Aug 2, 1982	60	0.41	0.41	3.8	Relapse
Aug 4, 1982	ND	ND	ND	ND	EF = 54%, final daunorubicin dose
Sept 14, 1982	ND	ND	ND	3.6	Day 1, high-dose cyclophosphamide
Sept 16, 1982	110	0.30	0.41	3.7	Day 3
Sept 20, 1982	66	0.40	0.42	3.6	Day 7
Sept 21, 1982	ND	ND	ND	4.9	Day 8, AMSA dose 1 EF = 41%
Sept 22, 1982	62	0.49	0.50	4.0	Day 9, AMSA dose 2
Sept 23, 1982	60	0.47	0.47	3.8 3.3	Day 10, AMSA dose 3; ventricular tachycardia
Sept 24, 1982	58	0.53	0.52	ND	Day 11, etoposide
Sept 27, 1982	85	0.43	0.51	3.5	
Oct 2, 1982	100	0.36	0.45	4.3	
Oct 8, 1982	72	0.39	0.44	3.2	Relapse

* QTc interval = measured QT interval/$\sqrt{\text{RR interval}}$. In 95% of children, the QTc is 0.45 sec (ref 9). All values represent mean of measurements. ND = not done. EF = cardiac ejection fraction by nuclear imaging.

to rat myocytes (11). High cumulative doses of anthracyclines and large single doses of cyclophosphamide are known to cause myocardial injury (12-14) and may have made this patient more susceptible to AMSA cardiotoxicity.

AMSA may itself cause QTc prolongation. Alternatively, other factors causing QTc prolongation [eg, hypocalcemia, phenothiazines, lithium, tricyclic antidepressants, antiarrhythmia agents, mitral valve prolapse, acute myocardial injury, and hypokalemia (15)] might act synergistically with AMSA to increase the risk of cardiac dysrhythmias and should perhaps be avoided in patients receiving AMSA. Furthermore, if QTc prolongation is in fact AMSA-induced, the appearance of AMSA-related QTc prolongation may be an ominous sign, leading to a greatly increased chance of serious cardiac dysrhythmias.

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Mitolactol Chemotherapy for Malignant Melanoma: A Phase II Study[1]

L. T. Malden,[2] A. S. Coates,[2,*] G. W. Milton,[3] W. H. McCarthy,[3] J. A. Levi,[4] R. L. Woods,[4] M. J. Byrne,[5] P. M. Reynolds,[5] R. M. Fox,[2] D. W. Hedley,[2] D. Raghavan,[6] and M. H. N. Tattersall[2]

Fifty-one patients (43 evaluable) with malignant melanoma were treated with mitolactol in an intermittent oral schedule. There were four objective tumor responses, all occurring in patients previously treated with dacarbazine. [Cancer Treat Rep 68:1045–1046, 1984]

PATIENTS AND METHODS

Existing chemotherapy for advanced malignant melanoma is unsatisfactory. We studied mitolactol (DBD), a halogenated hexitol alkylating agent previously reported (1,2) to have some antitumor activity against the disease. Between July 1980 and September 1982, 51 patients with histologically confirmed malignant melanoma with clinical evidence of residual, recurrent, or metastatic disease received DBD in an intermittent oral dosage schedule starting at 200 mg/m^2 daily × 10, repeated every 28 days. Doses were escalated to 250 mg/m^2 (12 patients) or to 310 mg/m^2 (two patients) where possible. Two heavily pretreated patients were started at a dose of 135 mg/m^2 and one was escalated to 170 mg/m^2.

Tumor responses and toxicity grades were assessed by standard World Health Organization (WHO) criteria (3). Eight patients were considered inevaluable for response (three with early deaths, two with major protocol violations, one withdrawal, and two for whom no data were available), leaving 31 male and 12 female patients evaluable. The mean age was 46 years (range, 18–74). Twenty-five patients had received prior chemotherapy as follows: dacarbazine (23 patients), lomustine (five), amsacrine (three), and cisplatin (three). Twelve patients had received more than one drug. Ten patients had received prior radiotherapy, 13 had received tamoxifen, and four had received prior BCG immunotherapy. Other patient characteristics are shown in table 1.

RESULTS

Four objective tumor responses were observed among 43 evaluable patients treated with DBD: two complete and two partial. All responding patients had a performance status (Eastern Cooperative Oncology Group [ECOG]) of 0 and had received dacarbazine, to which one patient had shown a tumor response. One patient had also received lomustine but without tumor response. Further clinical data on the four responders are shown in table 2.

In the remaining patients there were no significant tumor responses noted at any of the multiple sites involved. Patients with stable disease had disease progression after 6–32 weeks (median, 8). The median survival of treated

TABLE 1.—Clinical data on 43 evaluable patients

Characteristic	No. of patients
Performance status (ECOG scale)	
0	20
1	12
2–3	7
Sites of disease	
Cutaneous	27
Lymph node	13
Lung	19
Liver	6
Bone	5
Other	4

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[2]Ludwig Institute for Cancer Research (Sydney Branch), University of Sydney, Australia.

[3]The Melanoma Unit, Sydney Hospital, Australia.

[4]Department of Oncology, Royal North Shore Hospital, St. Leonards, Australia.

[5]Department of Medical Oncology, Sir Charles Gairdner Hospital, Nedlands, Australia.

[6]Department of Oncology, Royal Prince Alfred Hospital, Sydney, Australia.

[*]*Reprint requests to:* A. S. Coates, MD, Ludwig Institute for Cancer Research (Sydney Branch), University of Sydney, Blackburn Bldg, Sydney, New South Wales, 2006, Australia.

TABLE 2.—Clinical data on 4 patients responding to DBD

Patient No.	Age (yrs)/ sex	Response*	Response duration (wks)	Site of disease	Prior treatment
1	49/F	Partial	76+	Lung	Dacarbazine/lomustine
2	30/M	Partial	24	Lymph node	Dacarbazine/BCG
3	48/M	Complete	14	Lymph node	Dacarbazine/BCG
4	33/F	Complete	42	Cutaneous and breast	Dacarbazine

*WHO criteria (ref 3).

patients was 22 weeks (range, 4-112). Four patients progressed with cerebral metastases.

Toxicity

One hundred and twenty-eight cycles of treatment were administered (range, one to 12 cycles). The major toxic effect was myelosuppression. Blood cell counts adequate to assess hematologic toxicity were available for approximately one-half of these cycles. For these cycles, the median wbc count nadir was 3500/mm^3 at 21 days and the median platelet count nadir was 104,000/mm^3 at 28 days. Leukopenia < 4000 cells/mm^3 occurred in 44 of 62 cycles and thrombocytopenia < 100,000 platelets/mm^3 occurred in 15 of 65 cycles evaluable for toxicity (table 3).

There were three drug-related deaths, all occurring in the first cycle of treatment in association with profound thrombocytopenia. Two deaths occurred in previously irradiated patients and the third in a 70-year-old male.

Other toxic effects included nausea and vomiting in 11 patients, diarrhea in five, and mucositis in two.

CONCLUSION

We have found a tumor response rate of 9.3% (95% confidence limit, 3%-22%) for DBD. All of the responses occurred in patients previously treated with dacarbazine, suggesting a lack of cross-resistance between these agents.

The observed response rate is lower than that reported using a continuous schedule for DBD (1) but is higher than a schedule involving lower cumulative doses (2). All responses were observed in patients experiencing myelosuppression with therapy, suggesting that responses may be related to drug dosage and schedule.

Although the response rate is low, the activity demonstrated among patients refractory to dacarbazine may warrant investigation of combinations including these two agents.

TABLE 3.—Hematologic toxicity

	Wbc count		Platelet count	
WHO grade	Nadir (cells × 10^9/L)	No. of cycles	Nadir (cells × 10^9/L)	No. of cycles
0-1	≥ 3.0	43	≥ 75	55
2	2.0-2.9	15	50-74	7
3	1.0-1.9	4	25-49	2
4	< 1.0	0	< 25	1
Total No. of evaluable cycles		62		65

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Evaluation of Gallium Nitrate in Metastatic or Locally Recurrent Squamous Cell Carcinoma of the Head and Neck: A Southwest Oncology Group Study[1,2]

David A. Decker,[3] John J. Costanzi,[4] Joseph D. McCracken,[5] and Laurence H. Baker[3,*]

Twenty-one patients with metastatic or locally recurrent histologically proven squamous cell carcinoma of the head and neck were treated with gallium nitrate. Eligibility criteria included a lesion measurable in two dimensions, anticipated survival of at least 8 weeks, performance status ≤ 3 (Southwest Oncology Group criteria), wbc count ≥ 3000/mm^3, platelet count ≥ 100,000/m^3, serum creatinine ≤ 1.2 mg/100 ml, bilirubin < 2 mg/100 ml, and recovery from the toxic effects due to previous therapy. Primary sites were the tongue (five patients); larynx (four); head and neck (primary site not specified) (three); floor of mouth (two); and hard palate, soft palate, pyriform sinus, pharynx, glottis, major salivary gland, and nasopharynx (one each). Sixteen patients had received prior chemotherapy and 19 had had prior radiation therapy.

Gallium nitrate was administered at the dose and schedule described by Samson et al (1). Patients were hydrated prior to receiving gallium nitrate at a dose of 700 mg/m^2 in 250 ml of normal saline over 30 minutes every 2 weeks if the BUN was ≤ 20 mg/100 ml, serum creatinine was ≤ 1.2 mg/100 ml, wbc count was ≥ 3000/mm^3, and platelet count was ≥ 100,000/mm^3. Dose escalations were not allowed. Dose de-escalations were allowed for impaired renal function, myelosuppression, or diarrhea. An adequate trial consisted of two courses of therapy (over 4 weeks).

Patient characteristics are shown in table 1. Three patients died from progressive cancer within 4 weeks of beginning therapy. None of the 16 evaluable patients achieved an objective response. Six patients were observed to have stable disease for 4-5 weeks of therapy. The median survival was only 12 weeks (range, 1-83), suggesting that this group of patients was prognostically unfavorable.

All patients were evaluable for toxicity. Toxicity was limited to a transient episode of leukopenia (wbc count nadir of 1800/mm^3), a transient episode of thrombocytopenia (platelet count nadir of 50,000/mm^3), severe vomiting in one patient, mild diarrhea in two patients, and reversible renal failure with an elevated serum creatinine in two patients (ie, peak serum creatinine of 2.5 and 2.0 mg/100 ml). One of these latter two patients also had severe concurrent hypercalcemia which may have contributed to the renal failure.

We conclude that gallium nitrate, at the dose and schedule chosen, induces a true response rate of < 20% in

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	21
Fully evaluable	16
Partially evaluable	5
Early death	3
Refused further treatment	1
Concurrent irradiation	1
Median age in yrs (range): 54 (38-72)	
Sex	
Male	15
Female	6
Median performance score (range): 1 (1-3)	
Best response	
Complete/partial	0
Stable disease	6
Immediate disease progression	10
Median survival in wks (range): 12 (1-83)	
Toxicity	
Wbc count nadir (cells/mm^3)	
Median: 6200	
Range: 1800-16,200	
Platelet count nadir (cells/mm^3)	
Median: 294,000	
Range: 50,000-619,000	
Diarrhea (mild)	1
Vomiting (severe)	1
Renal (moderate)	2

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[3]Wayne State University Medical Center, Detroit, MI.
[4]University of Texas at Galveston.
[5]Brooke Army Medical Center, San Antonio, TX.
*Reprint requests to: Southwest Oncology Group (SWOG-7923), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

previously treated patients with advanced carcinoma of the head and neck, with a false negative error of not greater than 0.05 (2).

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Phase II Trial of PCNU in Advanced Renal Cell Carcinoma and Malignant Melanoma[1,2]

Jimmie H. Harvey, Frederick P. Smith, Mary Wyatt Bowers, John R. Neefe, Thomas P. Butler, John J. Gullo, Gerald Schertz, Julian Lokey, Philip S. Schein, and Paul V. Woolley[3,*]

PCNU is a new nitrosourea with several chemical features that might be associated with effective tumor activity: high alkylating activity and solubility properties favoring passage across the blood-brain barrier (1). Previous studies have demonstrated activity of chloroethylnitrosoureas in melanoma (2) and, in our own phase I trial of PCNU, one patient with metastatic renal cell carcinoma exhibited an objective tumor response (3). For these reasons, we undertook this phase II study of PCNU in advanced renal cell carcinoma and malignant melanoma.

PATIENTS AND METHODS

Thirty-seven patients with advanced renal cell carcinoma and 25 patients with metastatic malignant melanoma were entered in this phase II trial. Patient characteristics are summarized in table 1. PCNU was administered every 6 weeks as a 1-hour infusion at a dose of 75 mg/m^2 in previously treated patients and 100 mg/m^2 in previously untreated patients. Standard response criteria were used and treatment was continued until there was progression of disease. Six patients were unevaluable because of disease progression during the first cycle.

RESULTS

Of the 62 patients entered in this study, 34 with renal cell carcinoma and 22 with melanoma were evaluable for response (table 1). Fifteen of the melanoma patients had visceral disease. Responses in the melanoma group were as follows: one patient exhibited complete disappearance of both a lung nodule and a subcutaneous nodule and had subjective improvement of bone pain; a second patient had a partial response with reduction of skin nodules by $> 50\%$; and a third patient had a minor response with reduction of skin nodules by $< 50\%$. Among the patients with renal cell carcinoma, there was one partial response consisting of the disappearance of a pleural-based mass, but with persistence of 1-cm hilar lymph nodes. This response occurred after two doses of PCNU and lasted 6 months. The tumor in two patients remained stable during ≥ 3 months of therapy. In one patient, the disease remained stable for 3 months, when the patient died of a cardiac arrest. Two other patients remained stable for periods of 7.8 and 12+ months, and the latter patient remains on study. The median survival of all patients was 4.5 months (range, 0.5–18.0).

The principal toxic effect of PCNU was myelosuppression, primarily thrombocytopenia. Five patients developed platelet counts $< 100 \times 10^3/mm^3$, with the lowest being 22,000/mm^3. No patient required cessation of therapy because of myelosuppression and no correlation could be drawn between previous therapy and myelosuppression. One patient had a marked deterioration of renal function and was removed from study after three doses of PCNU, when creatinine clearance fell from a pretreatment level of 100 ml/minute to 50 ml/minute. No other nephrotoxic effect could be identified in this patient, and the toxicity was felt to be drug-related.

CONCLUSION

Despite theoretically attractive chemical properties, PCNU appears to have only marginal therapeutic activity in malignant melanoma and no activity in metastatic renal cell carcinoma. The doses of PCNU used in this trial are close to the maximum tolerated doses reported in phase I trials and it seems unlikely that further increases in dose would result in an improved response rate.

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[3]Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Washington, DC.

[*]*Reprint requests to*: Paul V. Woolley, MD, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, 3800 Reservoir Rd, NW, Washington, DC 20007.

TABLE 1.—Patient characteristics*

Characteristic	Renal cell carcinoma	Melanoma
Median age in yrs (range)	55 (30-77)	55 (28-74)
Median performance status (range)	2 (0-3)	2 (0-3)
Sex		
Male/female	27/10	18/7
Prior therapy		
None	8	6
Chemotherapy	11	5
Radiation therapy	9	5
Combined	6	6
Site of disease		
Lung	29	9
Liver	7	6
Brain	2	5
Bone	8	5
Skin	3	5†
Nodes	2	5
Other	4	6
Response (duration in mos)‡		
CR	0	1 (11)
PR	1 (11)	1 (4)
MR	0	1 (1)
Stable disease	3 (3, 8, 12)	1 (12+)
Progressive disease	30	18
Median survival in mos for all patients (range)	4.5 (0.5-18)	

* Unless otherwise specified, values = No. of patients.
†Distant from primary tumor.
‡CR = complete response; PR = partial response; and MR = minor response.

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Phase II Trial of Amsacrine in Children With Advanced Metastatic Neuroblastoma[1]

Bruno De Bernardi,[2] Guido Pastore,[3,*] Alberto Garaventa,[2] Giovanni Calculli,[4] Modesto Carli,[5] Adriana Ceci,[6] Luca Cordero di Montezemolo,[3] Clotilde Pianca,[7] Carlo Romano,[8] and Antonio Russo[9]

For the Italian Cooperative Group on Neuroblastoma

Amsacrine, an acridine derivative inhibiting DNA replication (1,2), has demonstrated activity in patients with advanced leukemia (3) and lymphoma (4) and is modestly active against some solid tumors (5). However, experience with pediatric malignancies is scarce.

MATERIALS AND METHODS

From January 1981 to June 1982, 21 children with refractory neuroblastoma were treated with amsacrine. Eligibility requirements were the following: age < 16 years, measurable disease, normal renal and liver functions, and adequate blood cell counts (wbc count > 3000/μl and platelet count > 100,000/μl).

Amsacrine (supplied by Warner-Lambert/Parke-Davis, Ann Arbor, MI) was administered iv at a dose of 60 mg/m²/day for 5 consecutive days, as a 60-minute infusion, and was diluted to a volume of 300 ml/m² in 5% dextrose and water. The cycle was repeated after 21 days and the dose was escalated to 75 mg/m² if the patient had adequate blood cell counts at that time (*see* above). In case of objective tumor response, amsacrine was given as maintenance therapy at the monthly dose of 60 mg/m²/day for 2 consecutive days.

RESULTS AND DISCUSSION

Of the 21 children registered, 20 were evaluable (table 1). One patient was disqualified because of inadequate records. No patient experienced either a complete or a partial response. Four patients had a minor response (a < 50% reduction of one or more but not all tumor lesions). Responding metastatic sites were: cervical nodes (one patient) and bone marrow (all four patients). Responses were recorded following the first amsacrine cycle, except in one patient, in whom clearing of the bone marrow did not take place until after two cycles of the

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total evaluable	20
Age in yrs	
Median: 3	
Range: 11 mos–6 yrs	
Sex	
Males	10
Females	10
Previous therapy	
Chemotherapy	20
Radiotherapy	7
On/off therapy at time of present study	18/2
No. of previous relapses	
None	8
1	4
⩾ 2	8
Sites of metastatic involvement	
Bone	14
Bone marrow	11
Abdomen	7
Lymph nodes	3
CNS	3
Liver	2
No. of evaluable tumor lesions	
1	1
2	7
⩾ 3	12

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[2]"Giannina Gaslini" Children's Hospital, Genova, Italy.
[3]Department of Pediatrics, University of Torino, Italy.
[4]Civic Hospital for Children, Brescia, Italy.
[5]Department of Pediatrics, University of Padova, Italy.
[6]Department of Pediatrics, University of Bari, Italy.
[7]"Bambino Gesù" Children's Hospital, Rome, Italy.
[8]Civic Hospital, Bergamo, Italy.
[9]Department of Pediatrics, University of Catania, Italy.
[*]*Reprint requests to:* Dr. Guido Pastore, Clinica Pediatrica dell' Università, Piazza Polonia 94, 10126-Torino, Italy.

drug. Of the four responders, the duration of response was 3 months in one patient but < 1 month in the other three. Sixteen children had either no change of their disease during the two amsacrine cycles (seven patients) or had evidence of progressive disease following the first (three) or the second (six) cycle.

The major dose-limiting toxic effect was myelosuppression. Nine children had a wbc count < 1000/μl (four, < 500/μl). Severe thrombocytopenia (< 20,000 cells/μl) developed in four patients. Fourteen children showed hematologic recovery allowing for dose escalation from 60 to 75 mg/m^2. Elevations of BUN (> 40 mg/100 ml) and serum creatinine (> 1.5 mg/100 ml) were detected in four patients. Elevated liver transaminases were observed in two patients. Nonspecific ECG abnormalities were noted in two children, but did not require specific treatment and did not interfere with continuation of amsacrine therapy. During leukopenia nadir, three children developed sepsis (lethal in two) and one developed an interstitial pneumonia (also lethal).

Our data indicate that amsacrine has minimal activity against advanced metastatic neuroblastoma. Toxicity is often severe and, in some cases, life-threatening. Further trials are not warranted at this time.

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Phase II Evaluation of Metoprine in Advanced Pancreatic Adenocarcinoma[1]

Cora N. Sternberg,[2] Gordon B. Magill,[2,*] Peter P. Sordillo,[2,3] Edgar W. Cheng,[2] and Nancy Kemeny[2,4]

Metoprine (DDMP) is a substituted 2,4-diaminopyrimidine and an inhibitor of dihydrofolate reductase. After entering clinical trial in 1953, antitumor activity was demonstrated in patients with acute leukemias but the toxicity was prolonged with a daily dosage schedule (1). Resurgence of interest is due to recognition of the activity of DDMP against methotrexate-resistant tumors. Also, it is a potent lipid soluble compound which can gain rapid entry into body compartments, such as the pancreas, from which more polar anticancer drugs are excluded (2). Phase I studies of DDMP in advanced cancer found thrombocytopenia to be dose-limiting at 65 mg/m^2 and CNS toxicity to be dose-limiting at 300 mg/m^2 (3). Responses were observed in patients with mycosis fungoides, non-Hodgkin's lymphoma, and adenocarcinoma of unknown origin. A phase II trial in soft tissue sarcomas at Memorial Sloan-Kettering Cancer Center resulted in several impressive responses (4). A similar trial against pancreatic adenocarcinoma was therefore initiated at Memorial Hospital and forms the basis of this report.

MATERIALS AND METHODS

Twenty-seven patients with histologically confirmed adenocarcinoma of the pancreas were entered in the study. All patients had objectively measurable disease that was not amenable to surgical resection. Criteria for entry included: wbc count $\geq$ 4000/mm^3, platelet count $\geq$ 150,000/mm^3, BUN $\leq$ 30 mg/100 ml, creatinine $\leq$ 1.5 mg/dl, bilirubin $\leq$ 2 mg/100 ml, no recent or concomitant radiation therapy, and Karnofsky performance status $\geq$ 50%. Informed consent was obtained for all patients. Characteristics of 24 adequately treated patients are detailed in table 1. Three patients were considered inadequately treated because they had received only one dose of DDMP. The starting dose of DDMP was 50 or 40 mg/m^2 orally every 2-3 weeks for previously untreated or previously treated patients, respectively. Doses were escalated or de-escalated by 10 mg/m^2-increments based on nadir wbc counts. An adequate trial was defined as at least two doses or myelotoxicity (wbc count < 3000/mm^3, platelet count < 100,000/mm^3) in the presence of progressive disease. Response criteria were as previously published (5).

TABLE 1.—Patient characteristics*

Total evaluable	24
Median age in yrs (range)	59 (47-76)
Median performance status (range)	80 (50-90)
Males:females	16:8
Prior chemotherapy	12
No prior chemotherapy	12
Sites of metastases	
Regional nodes	11
Liver	17
Abdominal mass	9
Peritoneal	3
Pulmonary	2
Bone	1
Subcutaneous	1
Radiologic assessment	
CT of abdomen	23
Ultrasound of abdomen	9
Liver radionuclide scan	4
Chest x-ray	2
Toxic effects	
Hematologic	
Median wbc count nadir (range)†	5800 (800-12,800)
Median platelet count nadir (range)†	96,000 (14,000-374,000)
Median hemoglobin nadir (range)‡	9.7 (5.3-12.6)
Stomatitis	7
Nausea	4
Vomiting	2
Diarrhea	2

* Unless otherwise specified, values = No. of patients.
†Cells/mm^3.
‡g/dl.

[1]Received Aug 10, 1983; revised Jan 26, 1984; accepted Feb 21, 1984.
[2]Department of Medicine, Solid Tumor Service, Memorial Sloan-Kettering Cancer Center, New York, NY.
[3]Recipient of a Junior Faculty Fellowship Clinical Award from the American Cancer Society.
[4]We thank Mrs. Sandra Sanchez-Muller for secretarial assistance in preparation of this manuscript.
Reprint requests to: Gordon B. Magill, MD, Department of Medicine, Solid Tumor Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

RESULTS

There were no complete or partial responses observed. One patient had complete shrinkage of liver metastases but the primary tumor continued to progress on computerized tomographic (CT) scan. The median time from diagnosis to treatment was 3 months (range, 0.2-20.6). Thrombocytopenia (platelet count < 100,000/mm^3) was the main hematologic toxic effect and was observed in 13 patients. Four patients received leucovorin and two required platelet transfusions. Stomatitis (mild-moderate), which was observed in seven patients, was the second most prominent problem. Gastrointestinal toxic effects were minimal and consisted only of occasional, transient nausea. There was no evidence of skin rash, headaches, or mental confusion (table 1).

DISCUSSION

Chemotherapy for pancreatic adenocarcinoma has generally been disappointing. With the advent of more sophisticated radiologic assessment of disease status (eg, CT scan), this entity is now more amenable to clinical trials. Twenty-four patients with pancreatic adenocarcinoma were evaluated in a phase II trial of DDMP. Toxic effects consisted mainly of thrombocytopenia. No meaningful tumor regression was observed (95% confidence, 0%-14% for the entire group and 0%-17% for the previously untreated group). These data indicate that DDMP has no value as a single agent in the treatment of adenocarcinoma of the pancreas.

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Phase II Trial of Diaziquone in Advanced Renal Adenocarcinoma[1,2]

Mogens Hansen,[3,*] Walter M. Gallmeier,[4] Jan Vermorken,[5] Eduard Holdener,[6] Heine H. Hansen,[7] Josette Renard,[8] and Marcel Rozencweig[9]

For the Early Clinical Trials Group of the European Organization for Research on Treatment of Cancer[10]

Diaziquone (AZQ) is a quinone derivative that exhibits significant activity against a number of experimental murine tumors (1). This report summarizes our phase II findings in renal adenocarcinoma.

MATERIALS AND METHODS

All patients had histologically documented renal adenocarcinoma which was unresectable, recurrent, or metastatic; all had nonirradiated measurable lesions. Normal blood cell counts, serum creatinine, and serum bilirubin were required. Informed consent was obtained according to legal and institutional requirements.

AZQ was administered as a 60-minute iv infusion at a dose of 27 mg/m^2 every 4 weeks. Provisions for dose modifications according to myelosuppression have been described previously (2). Response criteria were as recommended by the World Health Organization (WHO) (3).

RESULTS

Of 33 patients entered, 29 were evaluable (table 1). All but two evaluable patients received at least two courses of AZQ. There were 14 good-risk patients with no prior radiotherapy or chemotherapy and a performance status of 0–1 who received two to eight courses of AZQ.

One patient with pulmonary metastases achieved a partial response for 3.5 months. Two patients had stable disease for 25 and 34 weeks, respectively, whereas the remaining 26 patients had progressive disease within four courses of AZQ.

Among the 27 patients who received more than one course, 17 patients had the initial dose increased in subsequent courses, while the starting dose remained unchanged in seven patients and was decreased in three.

The good-risk patients had hematologic toxicity varying from WHO grades 1 to 4 (table 2).

Nonhematologic toxic effects consisted essentially of mild to moderate nausea and vomiting, which were seen in 13 patients. Hemorrhage and infection were encountered in two patients each. There were single episodes of diarrhea, stomatitis, and rash. No other adverse reactions were noted.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Evaluable	29
Median age in yrs (range): 58 (45–74)	
Median performance status (range): 1 (0–2)	
Prior therapy	
None	18 (2)*
Radiotherapy	7† (1)*
Chemotherapy	3 (1)*
Radiotherapy and chemotherapy	1‡
Indicator lesions	
Lung	15†
Lung and others	5
Soft tissue	5
Soft tissue and others	3
Bone	1

* No. of patients with prior hormone therapy is shown in parentheses.
†1 patient achieved a partial response.
‡Treatment initiated at a dose of 20 mg/m^2.

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[2]Supported by contract N01-CM-53840 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]Medical Department C, Bispebjerg Hospital, Copenhagen, Denmark.
[4]Medizinische Klinik, Klinik Nurnberg, Germany.
[5]Akademisch Ziekenhuis der Vrije Universiteit, Afdeling Onkologie, Amsterdam, The Netherlands.
[6]Medizinish Klinik C, Kantonsspital, St. Gallen, Switzerland.
[7]Finsen Institute, Copenhagen, Denmark.
[8]EORTC/Data Center, Brussels, Belgium.
[9]Institut Jules Bordet, Service de Médecine et Laboratoire d'Investigation, Clinique H. Tagnon, Brussels, Belgium.
[10]Other participants in the study include: *P. Siegenthaler*, Hôpital des Cadolles, Neuchatel, Switzerland; *E. Newlands*, Charing Cross Hospital, Fulham Palace Hospital, London, England; *V. Bramwell*, Christie Hospital and Hold Radium Institute, Manchester, England; *F. Cavalli*, Ospedale San Giovanni, Bellinzon, Switzerland; *H. Høst*, Det Norske Radiumhospital Oslo, Norway; *P. Obrecht*, Kantonsspital, Basel, Switzerland; and *M. Clavel*, Centre Léon Bérard, Lyon, France.
Reprint requests to: Mogens Hansen, MD, Medical Department C, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark.

TABLE 2.—Hematologic toxicity

WHO grade	Wbc count		Platelet count		No. of patients with WHO grade for both wbc and platelet counts
	x 10^3 cells/μl	No. of patients	x 10^3 cells/μl	No. of patients	
0	⩾ 4.0	7	⩾ 100	12	6
1	3-3.9	5	75-99	3	4
2	2-2.9	8	50-74	4	6
3	1-1.9	5	25-49	8	9
4	< 1.0	4	< 25	2	4

DISCUSSION

Only one of the 29 patients with renal adenocarcinoma was found to achieve partial response during AZQ treatment. These findings corroborate negative data reported by others (4). Our study included 14 ambulatory and previously untreated patients who had adequate trials. It is thus reasonable to conclude that AZQ has no role in the treatment of advanced renal adenocarcinoma.

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Facial Burning From Cyclophosphamide

High doses of cyclophosphamide are commonly used in preparing patients for bone marrow transplantation. We report here a newly described side effect of high-dose cyclophosphamide: burning in the peri-oral region, around the nares, and in the throat.

Patients in the Adult Bone Marrow Transplantation Unit at the University of Minnesota with chronic myelocytic leukemia (CML), acute myelocytic leukemia (AML), and acute lymphocytic leukemia (ALL) are treated prior to transplant with 60 mg/kg of cyclophosphamide per day for 2 days, followed by total-body irradiation. A fluid flush precedes and follows the 2-hour infusion of cyclophosphamide to prevent hemorrhagic cystitis.

In July and August of 1983, three patients treated with this regimen experienced unusual symptoms. A 29-year-old male in first remission from AML experienced burning around the mouth, throat, and nose during the cyclophosphamide infusion. This resolved after the drug infusion ceased. A 40-year-old female in chronic phase of CML had pain in her nose during the first day of cyclophosphamide infusion. During the second day of infusion, she experienced more severe burning of her mouth, face, tongue, and nose and could not open her mouth. These symptoms continued for > 1 hour after the cyclophosphamide infusion ended. Her calcium had fallen from 8.7 to 7.5, but she had no Chvostek's or Trousseau's sign. She was treated with calcium chloride, but it was unclear that the calcium hastened her recovery. The final patient was a 20-year-old female in her second remission from ALL. She had 20 minutes of mild burning sensation over her nose and up to her forehead. Her calcium level was normal at that time.

From July through the beginning of December 1983, we have performed transplants in 14 adult patients for leukemia. Five patients, including the three mentioned above, have experienced similar reactions of a burning sensation in the facial region.

The only prior report of similar side effects was in patients who received a bolus of cyclophosphamide > 4 seconds (1). Those patients experienced a fleeting posterior pharyngeal sensation lasting < 14 seconds. In contrast, our patients received a higher dose of cyclophosphamide over a longer period and the symptoms persisted up to several hours. Mead Johnson Laboratories (personal communication) has on file two cases of nasopharyngeal burning and four cases of conjunctival burning from cyclophosphamide.

There is no clear explanation for this observation. Cyclophosphamide can cause hypocalcemia in patients who develop tumor-lysis syndrome associated with hyperphosphatemia (2), but there was no evidence for this in our patients. Cyclophosphamide may also depress anticholinesterase activity (3) leading to neuromuscular irritability, but the relationship of this to our observation is uncertain.

In conclusion, in our experience, cyclophosphamide in high doses not uncommonly causes transient burning in the facial region, and this side effect should be monitored.

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Peter T. Silberstein
Gregory M. Vercellotti

Department of Medicine
University of Minnesota
Box 480 Mayo Memorial Bldg
420 Delaware St, SE
Minneapolis, MN 55455

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Serial Interferon Alpha$_2$ Levels in Serum and Cerebrospinal Fluid

Interferon has undergone many clinical trials, both as an antiviral and an antineoplastic agent. A perplexing and frequently observed side effect has been central nervous system toxicity. Clinically, this toxicity has been observed as drowsiness, inability to concentrate, disorientation to time and place, visual hallucinations, expressive aphasia, peripheral paresthesias, and upper motor neurone lesions (1,2). Rohatiner et al (1) and Smedley et al (2) have demonstrated abnormal electroencephalographic changes in cancer patients being treated with interferon. Both the clinical and electroencephalographic changes appear to be reversible.

TABLE 1.—Levels of r-IFN-$_{\alpha 2}$ in serum and CSF (timed from onset of infusion)

Time	Radioimmunoassay		Bioassay	
	Serum (units/ml)	CSF (units/ml)	Serum (units/ml)	CSF (units/ml)
0	< 5	< 2.5	nd*	< 37.5 (< 37.5)*
15 mins	3782	—	4800 (4800)*	— —*
30 mins	3065	< 2.5	2400 (2400)	< 37.5 (< 37.5)
1 hr	1611	3.1	1200 (1200)	< 37.5 (< 75)
2 hrs	370	3.4	600	< 75 (< 75)
4 hrs	107	3.2	300	< 75
8 hrs	22	3.8	600	< 75
12 hrs	10.8	< 2.5	600	< 75
24 hrs	5.5	< 2.5	300	< 75
26.5 hrs (Day 2)	963	4.4	600	< 75 (< 37.5)

* Values in parentheses = results of duplicate sample; dashes = no sample obtained; nd = not determinable.

While conducting a trial of recombinant alpha interferon (r-IFN-$_{\alpha 2}$; Schering Corp Labs), we had the opportunity to obtain serial samples of serum and cerebrospinal fluid (CSF) for interferon analysis from one patient, a 40-year-old woman treated for disseminated breast cancer. Prior to systemic administration of r-IFN-$_{\alpha 2}$, an Ommaya reservoir had been positioned into the right frontal horn of the lateral ventricle of the brain. She was treated with 27 million units of interferon infused for 30 minutes daily on 5 successive days at 3-week intervals. On Day 1, samples of serum and CSF were drawn at time 0, 15 and 30 minutes, and at 1, 2, 4, 8, 12, and 24 hours from the onset of infusion. Additional samples were drawn on Day 2, 2 hours following completion of a second infusion of r-IFN-$_{\alpha 2}$ at the same dose.

All samples were analyzed by Schering Corporation Laboratories, using a bioassay based on protection against viral cytopathic effect (CPE). Human foreskin cells were incubated with increasing dilutions of standard and unknown interferon preparations, then infected with mouse encephalomyocarditis virus, and later examined for virus CPE. The 50% end point of protection was determined by visual inspection. Each unknown was compared to a standard to obtain the final titer. The minimum sensitivity of this assay is < 75 units/ml. In spite of high serum levels achieved in this patient, the bioassay failed to detect interferon in the CSF (table 1).

All samples were also analyzed by radioimmunoassay. This technique employs ^{125}I NK-2 monoclonal antibody to human alpha interferon (Hu IFN$_\alpha$) and polystyrene beads with sheep anti-Hu IFN$_\alpha$ absorbed to them. This assay has a minimum sensitivity in CSF of 2.5 and 5.0 units/ml in serum. CSF levels ranging from 3.1 to 3.8 units/ml were detected 30 minutes after completion of interferon infusion and persisted through the eighth hour (table 1). Of interest was the observation that the highest CSF level (4.4 units/ml) was obtained 2 hours following a second dose of interferon, suggesting a possible accumulation in CSF with repeated iv doses. Due to the patient's deteriorating condition, no further samples of CSF were obtained to verify this possibility. Clinical neurological symptoms attributable to interferon were also not observed in this patient.

Our results are similar to those of Rohatiner et al (1), Priestman (3), and Jordan et al (4). Although high levels are achievable in serum, little or no interferon is detectable in CSF. In spite of these low levels, central nervous system toxicity remains a prominent side effect of this agent. It is interesting to speculate whether even low levels of interferon measurable in CSF correlate with either neurological symptoms or electroencephalographic changes.

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Silvana Martino*
Amnuay Singhakowinta

Wayne State University
School of Medicine
Detroit, MI

March 6, 1984

Reprint requests to: Silvana Martino, DO, Harper-Grace Hospitals, Rm 520 Hudson, 3990 John R, Detroit, MI 48201.

Adriamycin, a Decade of Experience: A Review and a Look to the Future

Chicago, Illinois, September 22, 1984

This symposium will include presentations reviewing structure-activity relationships, pharmacology, analogs, methods to reduce toxicity, and the mechanisms of drug resistance for doxorubicin (adriamycin) and other anthracyclines. An overview of clinical applications of the anthracyclines in leukemia, lymphoma, sarcomas, and solid tumors will be given. The role of the anthracyclines in adjuvant trials will be summarized.

The University of Chicago Cancer Research Center and Adria Laboratories, Inc, will sponsor the symposium; John E. Ultmann is the director. The University of Chicago Pritzker School of Medicine certifies that this program is accredited by ACCME for 7 hours of Category I credit. A registration fee of $25 is required.

For further information, contact: Jacqueline Samuel, University of Chicago Cancer Research Center (Box 444), 5841 S Maryland Ave, Chicago, IL 60637.

Urologic Cancer Course

Boston, Massachusetts, October 1–3, 1984

This intensive, 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. This course will emphasize the multidisciplinary approach which constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. While teaching will be primarily provided through lectures, panel discussion of problem management cases will be an integral part of the course. The course will emphasize cancers of the prostate gland, kidney, bladder, and testis. A comprehensive syllabus will be provided.

Guest faculty will include: Drs. Lawrence H. Einhorn, Carl A. Olsson, Michael J. Peckham, and Patrick C. Walsh. The Harvard/Boston faculty includes leaders in urologic cancer from the Dana-Farber Cancer Institute, Brigham and Women's Hospital, Beth Israel Hospital, Massachusetts General Hospital, Joint Center for Radiation Therapy, Lahey Clinic, University Hospital, Boston, City Hospital, and Children's Hospital Medical Center.

The course fee is $350, with a reduced rate of $200 for residents, house staff, fellows, and nurses.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA (telephone: 617-732-1525).

Twenty-First National Reticuloendothelial Society Meeting

Montreal, Canada, October 14–17, 1984

The Scientific Program Chair of this meeting, to be held at the Meridien Hotel, is Dr. Dolph O. Adams (Duke University, Durham, NC) and the Local Host Chair is Dr. Emil Skamene (Montreal General Hospital, Canada). The scientific sessions will consist of a discussion workshop, symposia, minisymposia, targeted sessions, poster sessions, and a keynote address. Symposia topics include: Cell-Cell Interaction: A Basic Host Recognition System; Recent Advances in Neutrophil Biology; Human Macrophages and Disease; and Cellular and Molecular Biology of Interferon.

For further information, contact: Reticuloendothelial Society, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (telephone: 404-828-2601).

Treatment of Metastasis: Problems and Prospects

London, England, October 15-17, 1984

This international meeting to be held at the Strand Palace Hotel is open to all those who are interested, but early application is advised. Registration and abstract forms will be sent on request and should be returned with the registration fee of £50 (£30 for research students on grants) no later than June 30, 1984. The registration fee covers the provision of programs, abstracts, lunches, and refreshments throughout the meeting. The proceedings of the meeting will be published. All papers on the subject of the meeting for oral or poster presentation will be considered.

For further information, contact: K. Hellmann, DM, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, England.

Two Short Courses: "Basic Biostatistics" and "Epidemiology: Principles and Applications"

Boston, Massachusetts, October 15-19, 1984

The New England Epidemiology Institute will present two consecutive short courses at the Hyatt Regency Hotel. "Basic Biostatistics," *October 15-16*, will be taught by Drs. William Louv and Harland Austin. They will present an overview for those who wish to become conversant with the fundamental concepts of biostatistics. "Epidemiology: Principles and Applications," *October 17-19*, will be taught by Drs. Kenneth Rothman and Stephan Lanes. They will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and public health.

No previous study of epidemiology or biostatistics is required for either course. Registrants may receive CME credits (American Medical Association, Category I), CEU credits (American Board of Health Physics), and/or CM credits (American Board of Industrial Hygiene).

For further information, contact: New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

International Workshop on the Use of Rodent Tumors in Experimental Cancer Therapy

October 21-24, 1984

This workshop is strictly limited to 60 investigators active in experimental radiotherapy, chemotherapy, and immunotherapy. This workshop will attempt to reach agreement on practices to follow when working with tumors in laboratory animals. The agenda will cover the following topics: Assays of therapeutic effectiveness by tumor control (TCD_{50}), tumor regrowth, and excision assay (both in vivo and in vitro); Choice of suitable tumor lines and their maintenance; Suitable host animal strains; Suitability of xenografts; and Statistical principles and details.

Plans are being made to hold the meeting outside the US, but the final location has not yet been determined. However, travel and attendance will be grant-supported. Those interested in attending should apply by *June 15, 1984* including a letter summarizing reasons for wishing to attend, experience in this field, present position and location, and a list of up to five publications that are relevant.

For further information, contact: Dr. R. F. Kallman, Department of Radiology, Stanford University Medical School, Stanford, CA 94305.

Symposium on Cancer Chemosensitivity Assay

Long Beach, California, October 21-25, 1984

Long Beach Community Hospital's Llewellyn Bixby IV Hematology-Oncology Laboratory, under the direction of Nathaniel B. Kurnick, MD, is sponsoring this international symposium to be held at the Hyatt Regency Hotel.

The conference will provide a forum for the most active and pre-eminent investigators in the field of cancer chemosensitivity assay to share their knowledge, exchange ideas, and discuss new techniques and methodologies. It is accredited by the California Medical Association for 19 hours of category one continuing education credit.

With emphasis on methodology and applicability, topics will include: Radio-nuclide methods, Clonogenic and other tissue culture methods, Animal models and xenografts, and other methods, such as histologic (including dye exclusion), metabolic, cytologic, and membrane integrity.

The symposium will be of interest to clinical oncologists, hematologists, and surgeons and will offer newer investigators the opportunity to meet with the established researchers from various parts of the world and to present papers, abstracts, or posters during the afternoon sessions.

The $350 per participant registration fee includes conference registration, all printed materials including agenda and abstracts, a reception cocktail party, and a gala dinner.

For further information, contact: Mrs. Mickles (telephone: 213-498-1000, ext 3600).

Cincinnati Cancer Conference III

Cincinnati, Ohio, November 2–3, 1984

Lung cancer is the focus of this conference to be held at the Hyatt Regency in Cincinnati.

Speakers for the conference are Paul A. Bunn, MD, Lawrence Einhorn, MD, Harvey M. Golomb, MD, George M. Hahn, PhD, Heber MacMahon, MD, Mary J. Matthews, MD, Clifton Mountain, MD, Carlos Perez, MD, Stephen Stellman, PhD, and Ronald Stephens, MD.

The course director is Barth Hoogstraten, MD, and Bethesda Hospitals will sponsor the event.

For further information, contact: Thomas J. O'Connor, Medical Staff Education, Bethesda Hospitals, 619 Oak St, Cincinnati, OH 45206 (telephone: 1-513-569-6337).

Innovative Cancer Chemotherapy for Tomorrow

New York, New York, November 7–9, 1984

The sixth biennial symposium of the Chemotherapy Foundation will be held at the Barbizon Plaza Hotel under the auspices of the Division of Medical Oncology of the Department of Medicine, the Department of Neoplastic Diseases, and the Page and William Black Post-Graduate School of Medicine of the Mount Sinai School of Medicine (CUNY).

Dr. Ezra M. Greenspan, Clinical Professor of Medicine (Oncology), is chairman of the meeting, which will include recent developments in chemoimmunotherapy and biological response modifiers. Symposium registrants are eligible for approximately 20 AMA Category I credits.

For further information, contact: Director, the Page and William Black Post-Graduate School of Medicine, Mount Sinai School of Medicine, 1 Gustave L. Levy Pl, New York, NY 10029 (telephone: 212-650-6772).

NCI-INSERM Cooperation in Basic and Clinical Cancer Research

The US National Cancer Institute (NCI) and the French Institut National de la Santé et de la Recherche Médicale (INSERM) jointly sponsor an agreement to promote cooperation in basic and clinical cancer research. Limited funds are now available for this program to provide travel expenses and subsistence allowance for US scientists to work in France for periods of up to 1 year on appropriate collaborative research projects with French colleagues. In addition, similar funds are available to pay the living expenses for French scientists to work in the United States on collaborative cancer research projects with American colleagues. Each request for support will be reviewed for scientific merit by the American and French program committees. The deadline for applications for this year is *October 31, 1984*.

For further information, contact: Dr. Bruce Chabner, Director, Division of Cancer Treatment, National Cancer Institute, Bldg 31, Rm 3A52, Bethesda, MD 20205; *or* Dr. Christine Chirol, Hôpital Cochin, Bâtiment Gustave Roussy, 7e étage, 27 rue du Faubourg St. Jacques, Paris 14e, France.

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